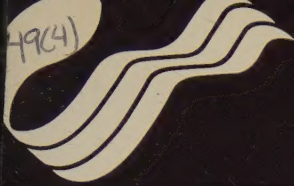
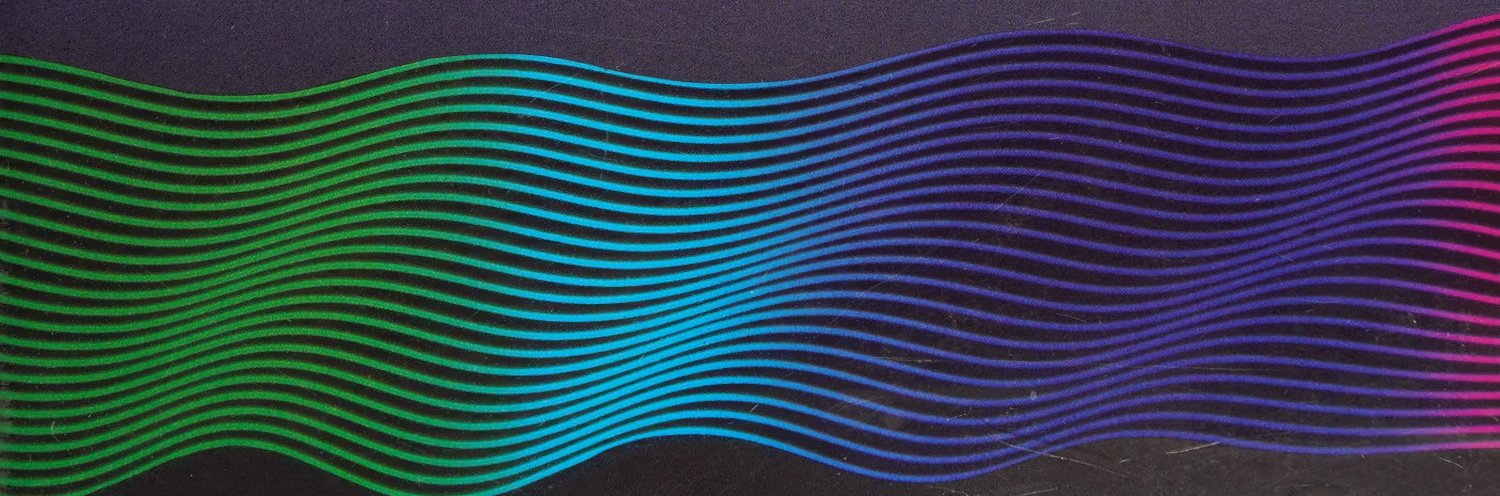


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Length-at-Age Analysis: Can You Get What You See?

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Mulligan, T. J., and B. M. Leaman. 1992. Length-at-age analysis: can you get what you see? Can. J. Fish. Aquat. Sci. 49: 632–643.

Observations at a single point in time of length-at-age (LAA) for a long-lived rockfish (*Sebastes alutus*) show that old fish are shorter than intermediate-aged fish. Fitting of a von Bertalanffy growth model to these data produces a systematic trend in the residual of observed versus calculated LAA. We examined how such LAA data can lead to erroneous conclusions about individual growth, and whether asymptotic growth can give rise to such data. We considered two hypotheses: (i) that a time trend in growth rate resulted in larger fish in more recent years and (ii) that there are multiple growth types, where growth and mortality rates are directly related. Using a general growth model that incorporated both (i) and (ii), we show that both hypotheses can generate data identical to those for the rockfish. A single set of LAA data is inadequate for describing individual growth; however, if sufficient data are available, model ambiguity can be resolved and reasonable parameter estimates obtained. Analysis of the rockfish data indicates that (ii) is more likely to explain the observations than (i). We show how fisheries on such species may preclude our understanding these biological relationships.

Les observations ponctuelles de la longueur selon l'âge (LA) chez une espèce de sébaste vivant longtemps (*Sebastes alutus*) indiquent que les poissons âgés sont plus courts que les poissons d'âge moyen. L'ajustement d'un modèle de croissance de von Bertalanffy à ces données donne lieu à l'apparition d'une tendance systématique des résidus pour les LA observées et calculées. Nous avons étudié comment de telles données sur la LA pouvaient être à l'origine de conclusions erronées quant à la croissance des individus et si une croissance asymptotique pouvait expliquer ce phénomène. Nous avons examiné deux hypothèses : i) une tendance temporelle du taux de croissance s'est traduite par de plus gros poissons au cours des dernières années et ii) il existe plusieurs types de croissance à relations directes entre les taux de croissance et de mortalité. À l'aide d'un modèle général de la croissance tenant compte de ces deux hypothèses, nous avons montré que toutes les deux permettaient de générer des données identiques à celles obtenues pour le sébaste. Un seul ensemble de données LA ne permet pas de décrire correctement la croissance individuelle, mais si un nombre suffisant de données est disponible, l'ambiguïté du modèle peut être éliminée et il est possible d'obtenir des estimations acceptables des paramètres. L'analyse des données sur le sébaste a montré que l'hypothèse ii) était plus apte à expliquer les observations que l'hypothèse i). Nous montrons aussi comment la pêche de telles espèces peut nuire à notre compréhension de ces relations biologiques.

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A frequent requirement of fish population studies is the estimation of growth parameters for individual fish. It is common practice to use length-at-age (LAA) data for this purpose. If we know how individual fish grow, we should be able to predict the size distribution at any age. Many examples from the literature illustrate this approach (Summerfelt and Hall (1987) contains recent reviews). Although the study of fish growth is not without controversy (Knight 1968; Roff 1980), many people believe that some form of determinate growth is appropriate for most fish species (Brett and Groves 1979). Specifically, we feel that some form of asymptotic growth is the correct description for the deepwater rockfish Pacific ocean perch (*Sebastes alutus*, called "perch" hereafter). Over the past several years we have made field collections of perch, and the LAA distribution for females of one population from the west coast of Canada is a result of this sampling program (Fig. 1). These data have been truncated to exclude fish of age <10 yr because of the possibility of sampling gear selectivity for these fish (Leaman 1991). This population is distinctive because the commercial fishery is a very recent phenomenon and has not yet affected demographic characteristics of the population. The gradual recruitment (50% of the cohort is vulnerable only at ages >10 yr) and the extensive age composition of our LAA

sample (up to age 80 yr) show that this sample is representative of the stock in an unexploited state.

The observed LAA distribution (Fig. 1) and the result of fitting a von Bertalanffy type growth function illustrates two points. First, there is an inordinate number of "outliers" (including the four points plotted with a different symbol) of extremely short length for the measured age. Second, the asymptotic growth function seems, at first glance, to provide a reasonable description of the data. However, closer analysis reveals a systematic bias in the mean residual for each age (Fig. 2). The trend line through these data shows that the residuals are predominantly positive between ages 15 and 40, while for ages >40, they are negative. This trend line is insensitive to the "outliers" mentioned above. Estimation of the trend line with the "outliers" removed results in a nearly identical line.

What is the explanation for this systematic trend? Physiological indicators such as fecundity or specific fecundity (eggs per gram body weight) do not support an argument for shrinkage with age (Leaman 1987, 1988). Since the same phenomenon also occurs with length-stratified sampling, the result does not appear to be a sampling artifact. In addition, studies indicate that the ageing method used for this species is valid (Bennet et al. 1982; Leaman and Nagtegaal 1987; Nedreaas 1990) and

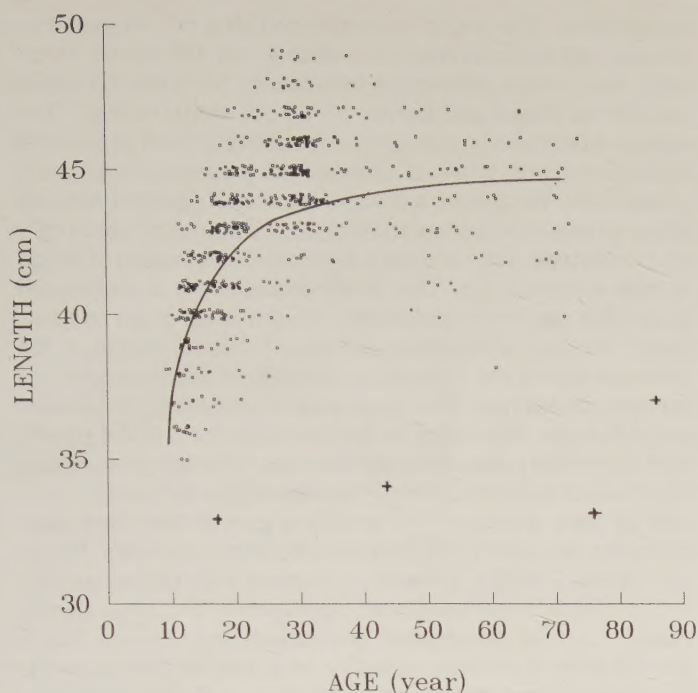


FIG. 1. LAA's for females ($N = 694$) from an essentially unexploited stock of Pacific ocean perch off the coast of British Columbia, Canada. The line represents a von Bertalanffy growth function which has been fit to these data. Fish lengths were measured to the nearest centimetre, but the plotted lengths have been offset slightly to help show the distribution of observations.

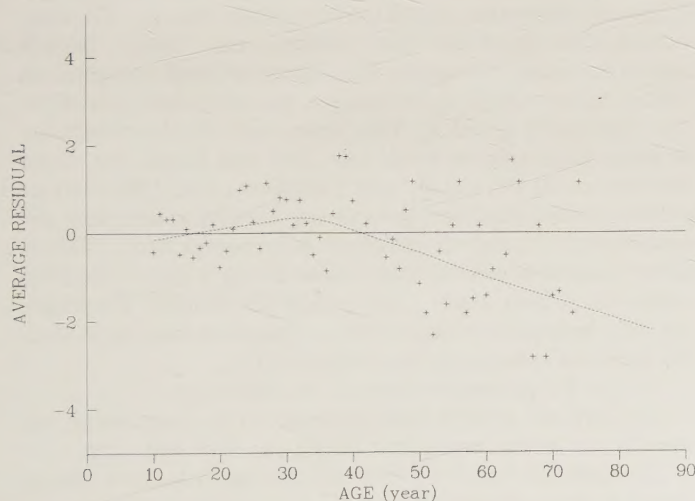


FIG. 2. Means by age of the residuals of observed versus estimated LAA for the data of Fig. 1. The line represents a LOWESS curve that has been fit to these data and shows underestimation of the length at younger ages with overestimation of the length at older ages.

that ageing errors would tend to underestimate the ages of older fish. This type of ageing error would mask, rather than emphasize, the effects we note. The size of the ageing error required (>30 yr) to explain the presence of these "outliers" is much larger than can be reasonably expected. Since the same type of LAA is seen from several independent sampling cruises, length measurement errors also seem unlikely. We believe that some form of asymptotic growth is the correct description for individual fish. Any type of asymptotic growth curve will give a similar result, namely, older fish tend to be shorter than the population's asymptotic length. Are these ideas compatible?

Can asymptotic growth of individuals result in a LAA distribution where older fish have a smaller mean length than younger fish?

One possible explanation is a growth type dependent (or indirectly, a growth rate dependent) mortality. This is similar to a size-dependent mortality, since fish that have grown faster will, in general, be larger. Some effects of a size-dependent mortality alone have been examined extensively (Ricker 1969). In particular, the bias introduced when back-calculating LAA from examination of only older fish ("Lee's phenomenon") has received much attention (Jones 1958; Ricker 1969). However, we suggest that growth and mortality rates are related physiologically, rather than mortality rate being related to size alone.

A second possible explanation for our perch LAA data is a long-term trend in growth. This is a time-dependent process. Older fish could have been born during a time when growth was less than it is at present. Therefore, younger fish in the current population would be larger at age than older fish would have been. Although this explanation is plausible, we are unaware of any reports of such trends in the absence of fishing pressure. Such trends have been reported for stocks with major historical fishing pressure (Boehlert and Yoklavich 1987; Smith et al. 1990).

Only recently have newer ageing techniques revealed that longevity is a feature of many more fish species than was previously supposed (Archibald et al. 1981; Summerfelt and Hall 1987). For example, samples of perch which were originally thought to range in age from 5 to 30 yr are now estimated to range from 5 to 80 yr (Stanley 1987). With former ageing techniques, these old, small fish would have increased the range of the length distribution of younger age groups.

In this paper we describe how estimating growth parameters from typical LAA data can lead to erroneous conclusions about growth of individuals. It can also lead to ambiguity in the determination of an appropriate growth model. We compare two growth models and show that asymptotic growth for individual fish, combined with *growth type dependent mortality*, could result in a LAA distribution similar to our perch data. We also show that a different explanation of growth, time-dependent growth parameters combined with *growth-independent mortality*, could yield a similar LAA distribution. We describe these growth processes for an unexploited population, rather than for a population with fishery induced responses. We demonstrate that a single set of LAA data for a population may be inadequate to describe the growth of individuals. Lastly, we show that unique model identification from these competing alternatives is possible and that reasonable parameter estimates can be obtained if sufficient data are available.

Materials and Methods

Our primary problem is the lack of direct observations of individual fish growth, yet that is what we are attempting to describe. We consider three levels of information about individual growth: repeated measurements of the same individuals over time (individual growth data), a series of measurements from samples of a given cohort over time (cohort growth data), and measurements of individuals from all cohorts present at one time (LAA data). Our data represent this final level, which is quite distantly related to the property we would like to describe, individual growth.

Most growth models attempt to describe individual growth data. If growth rate, mortality, and other parameters of these models are independent of time, size, etc., then the same model

may also describe the second and third levels of growth information. However, if some parameters exhibit these dependencies, then fitting a particular model to the LAA data will not yield the same information on growth as fitting the individual growth data. For many deepwater marine fish such as perch, mortality caused by capture means that individual growth cannot be directly observed and, therefore, model identification can be difficult or impossible.

Of the potential explanations of growth that could describe the perch LAA distribution, we consider two. We call the first "multiple pattern" (MP) growth. Fish that grow by this process have variable growth types within a cohort. As fish age, growth type dependent mortality causes changes in the mean LAA. Thus, MP growth is a growth type dependent process. The second potential explanation of the perch growth is "time-varying" (TV) growth. In this process, fish within a cohort have a common growth, but growth varies among cohorts. Thus, TV growth is a time-dependent process. We describe here a combined model that incorporates both of these growth processes, yet preserves asymptotic growth of individual fish. We then join this growth model with a model of a finite mixture of normal distributions to estimate all of the parameters of the combined model.

A Combined Model for LAA Analysis

In overview, the model relates growth rate, mortality rate, asymptotic length, and natal year. The MP and TV growth processes have specific assumptions.

For the MP process, these are as follows.

(1) Fish in the population do not grow identically; rather, each fish has one of a finite number (G) of discrete growth types (Fig. 3).

(2) Each growth type has a unique growth rate, asymptotic length, initial proportion of the cohort, and mortality rate (Fig. 3a). Faster growing fish reach a larger asymptotic length and have a higher mortality rate than slower growing fish. Thus, MP growth results in a higher fraction of the slower growing fish surviving to older ages.

(3) Fish comprising each cohort include a mixture of different growth types (Fig. 3b). The proportion of each growth type changes from one age group to the next, due to the different mortality rate of each type (Fig. 3c).

The MP model has conceptual roots in the direct relation between growth and survival commonly observed among fish genera (Beverton and Holt 1960). We extend this argument to the species level. We have linked mortality rate to growth type, rather than to size of the individual, and have also allowed variation within each growth type. This variation within the MP model permits some slow-growing individuals from the high-growth type to grow more slowly than some fast-growing individuals from a slower growth type. Thus, the MP model allows stochastic growth about mean values of growth rate, asymptotic length, and mortality rate within each growth type.

Genetically, growth rate is likely to be pleiotropic, and while there may be a finite number of growth types, the variation of growth rate is large and effectively continuous. However, this observed phenotypic continuum will have discrete genetic variation as its basis. The MP model reflects this discrete basis and also readily permits the estimation of biologically meaningful parameters. We examine a small number of growth types for simplicity, but there are no theoretical limits to the number of types.

A recent seminal paper by Parma and Deriso (1990) includes both a size-selective mortality and multiple growth types within

a population. This paper was published after our original submission and contains features similar to our MP model. However, some major differences between the MP growth process and that of Parma and Deriso (1990) are worth noting. They assume a continuous, normal distribution of growth parameters in the population; however, they restrict parameter variation to growth rate. We assume a discrete number of growth types and allow growth rate, asymptotic length, and mortality rate to vary by growth type. They assume a log normal distribution of length at their reference age. This distributional shape is maintained as the fish age, with predictable changes in its mean and variance. We can approximate any distribution of length at the reference age by the appropriate selection of initial proportions for each growth type. This shape may or may not be maintained as the fish age, depending on the functional form of the growth type dependent parameters. In this sense, we attempt to explain observations rather than simply accounting for the variance created by their presence. We specify a growth type dependent mortality, in contrast with their size-dependent mortality. Parma and Deriso's work is primarily concerned with fishing mortality, for which size dependence is appropriate. Our LAA sample comes from an unexploited population. We assume that a growth type dependent mortality is a natural phenomenon, occurring within a species, in the absence of fishing mortality.

Both our model and that of Parma and Deriso result in faster growing animals reaching a larger asymptotic size than slower growing animals. While intuitively obvious, this concept may appear inconsistent with some studies of von Bertalanffy growth parameters for a species among different areas, e.g. latitudinal growth studies. In such studies, there is often an inverse relation of the rate parameter, k , and the asymptotic size, L_{∞} . The conclusion often drawn from these studies is that "faster" growth results in smaller asymptotic size. However, such comparisons cannot be made without reference to the third parameter of the von Bertalanffy model, t_0 . This parameter is the theoretical age at which the organism would have had zero length and, when unconstrained, will usually take a negative value. Observations of the inverse relationship between k and L_{∞} are generally accompanied by a direct relationship between k and t_0 . This is usually due to a lack of observations at small sizes; hence the estimates of both k and t_0 are poorly determined. Therefore, the only biologically meaningful comparisons from such studies are those concerning the estimates of L_{∞} .

For the TV process we assume the following.

(1) Only one growth type is present in the population, but growth rate and/or asymptotic length can vary with time.

(2) Each fish follows a characteristic growth curve, determined in its natal year, throughout its lifetime.

By having growth type fixed at natal year, we do not imply that growth must be completely invariant throughout life, but simply that the dominant change in growth will be a time-dependent trend. In addition, since most of the growth occurs in the first few years of life, a slowly time-varying growth rate will be expressed primarily in growth of these young fish and have little effect on older fish. Biologically, growth rate and asymptotic length may be linked. Our model does not explicitly introduce this constraint in order to provide more flexibility in examining growth data.

The resulting LAA distributions from MP and TV growth are very similar (Fig. 4) and result in mean LAA's that decrease past some age. The major difference between the two LAA centroids shown in the figure is that this decrease is linear with age for TV growth, while for MP growth it is not. If the time dependence of the parameters in TV growth were nonlinear,

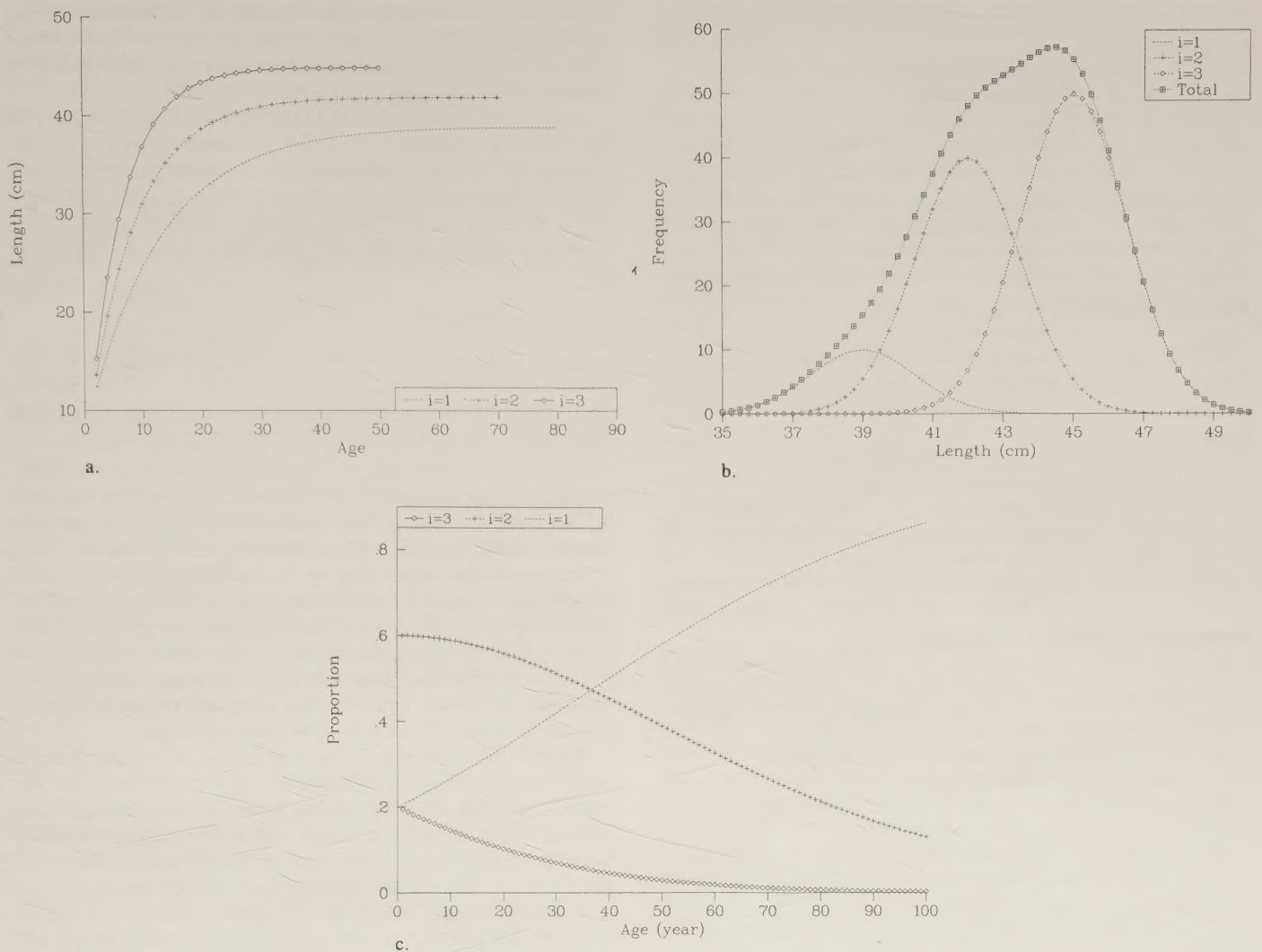


FIG. 3. Features of the MP growth model. (a) The LAA for a MP population with three growth types. The lines have been truncated when less than 10% of the original number of fish with this growth type remain alive. (b) Length distribution, at a given age, for each growth type of a three growth type population. The combined distribution for the whole population at this age is also shown. (c) The proportion of each growth type as a function of fish age.

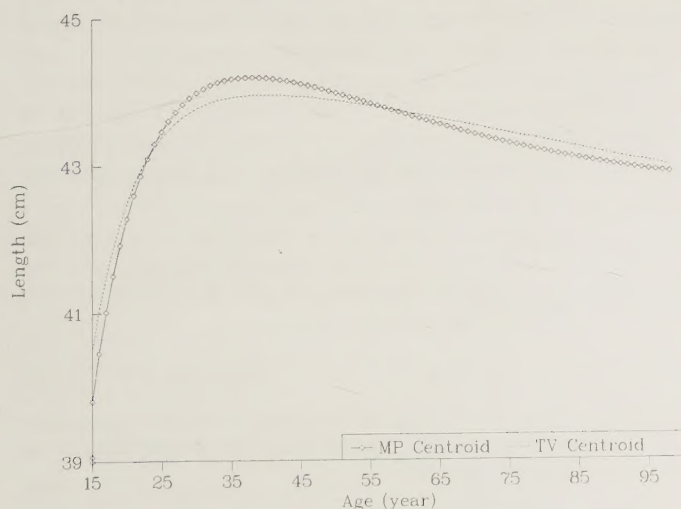


FIG. 4. Comparison of the shapes of LAA distributions for MP and TV growth.

the resultant growth patterns could be even more similar. Choosing between these growth processes, with limited observations, can be difficult. We will demonstrate that the choice can be straightforward if more extensive data are available.

Model Formulation

The central feature of the combined model is the description of how individual fish grow. We describe growth of individual fish by a von Bertalanffy function, as reformulated by Schnute (1981). This function provides statistically stable estimates, has been used widely, and offers a reasonable description of many data sets. We observed the length (ℓ), age (a), and natal year (T) for each fish in the LAA sample. (Table 1 contains a complete listing of notation used in the paper.) Our observations were acquired over more than 1 yr; hence, age and natal year are required to allow unique identification with respect to time.

Following Schnute's formulation, the length of the q th fish at age a_q is given by

$$(1) \quad \ell_q = L_0 + [L - L_0] \left[\frac{1 - K^{(a_q - a_0)}}{1 - K^{(a_{\max} - a_0)}} \right] + \epsilon_q$$

TABLE 1. Symbols used in the mathematical description of growth.

Indexing labels	
i	indexes growth type
j	indexes fish age
q	indexes fish number
r	dummy index
Ω	indexes natal year
Observed quantities for each fish in the sample	
a_q	age of the q th fish
l_q	length of the q th fish
T_q	natal year of the q th fish
Observed quantities for the sample as a whole	
$a_{\max}$	maximum age
N	number of fish in the sample
$T_{\min}$	minimum natal year
$T_{\max}$	maximum natal year
Constants	
a_0	reference age; set to 1
L_0	fish length at the reference age; set to 8
f	factor multiplying the penalty function; set to 0.01
G	number of growth types for MP type growth; variable, set by the user
Independent parameters for all models	
K	growth rate
K_M	incremental growth rate for MP-type growth
K_T	incremental growth rate for TV-type growth
L	asymptotic length
L_M	incremental asymptotic length for MP-type growth
L_T	incremental asymptotic length for TV-type growth
m	incremental mortality rate
σ	standard deviation of length distributions
Φ_i^2	proportion of fish of growth type i in the sample at the reference age
Parameters constructed from the independent parameters	
$K_i = K + (i-1)K_M$	
$K_{\Omega} = K + (\Omega-1)K_T$	
$K_{i\Omega} = K + (i-1)K_M + (\Omega-1)K_T$	
$L_i = L + (i-1)L_M$	
$L_{\Omega} = L + (\Omega-1)L_T$	
$L_{i\Omega} = L + (i-1)L_M + (\Omega-1)L_T$	
$m_i = (i-1)m$	

where ϵ_q is a normal deviate with mean 0 and standard deviation σ . Parameter L_0 is the length at some reference age, a_0 . This parameter requires that fish have finite length at real ages and avoids the artifacts associated with the von Bertalanffy parameter t_0 , as noted previously. A simple extension of (1) incorporates the TV process

$$(2) \quad \ell_q = L_0 + [L_{\Omega} - L_0] \left[\frac{1 - K_{\Omega}^{(a_q - a_0)}}{1 - K_{\Omega}^{(a_{\max} - a_0)}} \right] + \epsilon_q$$

where L_{Ω} and K_{Ω} are defined in Table 1 and Ω indexes natal year of the q th fish, i.e. $\Omega = (T_q - T_{\min})$. Table 1 shows that the individual values of the L_{Ω} 's (K_{Ω} 's) are linearly related and will either increase or decrease with Ω depending on the sign of the parameter L_T (K_T). This simple linear relationship among the L_{Ω} 's (K_{Ω} 's) allows us to specify individual parameter values for many natal years by the use of only two independent parameters, the constant parameter L (K) and the incremental param-

eter L_T (K_T). A more complex functional dependence for L_{Ω} and K_{Ω} is possible; we have restricted our models to this linear form for simplicity. Similar linear relations are used below to describe other families of parameters.

An alternative extension of (1) gives the MP growth process for i growth types ($i = 1, \dots, G$). For each growth type, we assume that the LAA data come from a normal distribution (g_i) centred about a growth type specific mean (μ_i) but sharing a common variance (σ^2). The μ_i follow asymptotic growth relations as a function of fish age. Thus, the lengths are described by

$$(3a) \quad \ell \sim N(\mu_i, \sigma^2)$$

where μ_i is given by

$$(3b) \quad \mu_i = L_0 + [L_i - L_0] \left[\frac{1 - K_i^{(a_q - a_0)}}{1 - K_i^{(a_{\max} - a_0)}} \right].$$

The L_i and K_i terms in (3b) are unique for each of the i growth types (Table 1). (MP growth with $G = 1$ gives the standard von Bertalanffy growth.) The values of L_i and K_i either increase or decrease with i , depending on the sign of L_M and K_M , respectively. Equations (3a) and (3b) incorporate the idea of multiple growth types but do not include the other component of MP growth, differential mortality. Since the MP growth model hypothesizes that mortality rate is specific to growth type, we must now develop a structure that permits changes in the relative proportions of these growth types with age.

We have not explicitly linked growth rate, asymptotic length, and mortality rate in the formulae of Table 1. Although the MP model embraces the concept that faster growing animals will attain a larger asymptotic length and have a higher mortality rate, the mathematical formulation of the MP model allows the flexibility to verify if this hypothetical linkage exists.

While the parameters of the TV growth process (2) can be estimated from the LAA data, parameter estimation for the MP growth process (3) requires estimation of the proportions of each growth type, P_i , at each age. Mixture models are widely used for this purpose and their statistical properties are well determined (Hasselblad 1966; Yakowitz and Spragins 1968). These models permit the estimation of the components of a mixture, e.g. the overlapping size distributions from different growth types at a given age. A combination of the MP growth model of (3) with the finite mixture model allows simultaneous determination of the P_i 's and the growth parameters.

Our solution for a finite mixture of normal distributions follows the development of Everitt and Hand (1981). The pdf for a fish of length ℓ_q coming from a mixture of G growth types, where each growth type has an individual pdf of g_i , is given by

$$(4a) \quad \sum_{i=1}^G P_i g_i(\ell_q)$$

where the constraints

$$(4b) \quad P_i \geq 0, \quad \sum_{i=1}^G P_i = 1$$

are automatically satisfied by defining the proportions P_i in terms of the parameters Φ_i :

$$(4c) \quad P_i = \frac{\Phi_i^2}{\sum_{r=1}^G \Phi_r^2}.$$

Equations (2)–(4) provide the three components of our combined growth model, i.e. MP growth, TV growth, and a mixture model for analyzing the mixture of MP growth types. We now synthesize these ideas and form a single likelihood function for parameter estimation.

Parameter Estimation

Estimates of the combined model parameters are obtained by maximizing the log likelihood function ($\mathcal{L}$). We begin with the log likelihood function for a classical mixture model of G normal distributions:

$$(5) \quad \mathcal{L} = \sum_{q=1}^N \ln \left[\sum_{i=1}^G P_i g_i(\ell_q, \theta) \right].$$

The model describes a mixed sample of size N drawn from G normal distributions (Fig. 3b). The objective is to determine simultaneously the proportions P_i of each growth type and the parameters which characterize the distributions g_i . The parameter vector θ describes the shape of the g_i , e.g. its components are the mean LAA's for different growth types and their associated standard deviations.

We expand (5) to incorporate both MP and TV growth by modifying the g_i 's, to incorporate the changes represented in (2) and (3), and the P_i 's, to include a growth type dependent mortality. The resultant log likelihood for each age (j) and natal year (Ω) combination is

$$(6a) \quad \mathcal{L}_{j\Omega} = \sum_{q=1}^N \ln \left[\sum_{i=1}^G P_{ij}(a_q, \Phi_i, m_i) g_{i\Omega}(\ell_q, \sigma, \mu_{i\Omega}) \right]$$

where the constraints

$$(6b) \quad P_{ij} \geq 0, \quad \sum_{i=1}^G P_{ij} = 1$$

hold for each value of j . Each age – natal year sample can be thought of as a separate mixture problem. For each j :

$$(7a) \quad P_{ij}(a_q, \Phi_i, m_i) = \frac{\delta(j, a_q) \Phi_i^2 \exp[-m_i(a_q - a_0)]}{\sum_{r=1}^G \Phi_r^2 \exp[-m_r(a_q - a_0)]}$$

where the Kronecker delta function, δ , is defined as

$$(7b) \quad \delta(j, a_q) = 1, \quad \text{if } j = a_q \\ = 0, \quad \text{if } j \neq a_q.$$

In (7a), each Φ_i^2 represents the proportion of growth type i present in the population at a reference age, a_0 , chosen here to be 1 yr. The P_{ij} 's contain an exponential mortality rate m_i that acts to change the proportion of each growth type as the fish age (Fig. 3c). For example, if the m_i 's become larger with i , then the ratio of P_{ij}/P_{2j} will increase with increasing fish age.

The $g_{i\Omega}$'s in (6a) are normal distributions, so that

$$(8a) \quad \ell \sim N(\mu_{i\Omega}, \sigma^2)$$

where $\mu_{i\Omega}$ follows the asymptotic growth relation

$$(8b) \quad \mu_{i\Omega} = L_0 + [L_{i\Omega} - L_0] \left[\frac{1 - K_{i\Omega}^{(a_q - a_0)}}{1 - K_{i\Omega}^{(a_{\max} - a_0)}} \right]$$

and describes the mean length of growth type i , natal year Ω fish as a function of age. The growth and mortality parameters

of (7) and (8) combine both MP and TV growth processes and are defined in Table 1.

The individual log likelihood functions $\mathcal{L}_{j\Omega}$ in (6a) are combined into an overall log likelihood function, $\mathcal{L}^*$. In (7a) the Φ_i 's are ill defined. Multiplication of the Φ_i 's by an arbitrary constant would leave the P_{ij} 's unchanged. To remove the degeneracy of the solution for the maximum of $\mathcal{L}^*$, a penalty function has been added to (9). The penalty function acts to force the maximum of $\mathcal{L}^*$ to occur at a specific value of the Φ_i 's. The form of the penalty function is consistent with the interpretation of Φ_i^2 as the proportion of the i th growth type at the reference age. The final expression for the log likelihood is given by

$$(9) \quad \mathcal{L}^* = \sum_{j=a_0}^{a_{\max}} \sum_{\Omega=T_{\min}}^{T_{\max}} \mathcal{L}_{j\Omega} + fN \left[\sum_{i=1}^G \Phi_i^2 - 1 \right]^2$$

where the constant f is assigned some fixed value that balances the penalty function, the last term in (9), with the first term.

Parameter estimates for our combined model were obtained by maximizing (9). A Polak–Ribiere conjugate gradient function minimization algorithm (Press et al. 1986) was used to minimize the negative of (9), using a custom FORTRAN computer program.

Simulation of Test Data

Simulated data were used to test our ability to detect the correct growth model. Therefore, the simulated samples did not conform to the constraints that might be expected for real samples. One of us independently wrote the length frequency simulator, whose output was then provided in blind tests to the parameter estimation program, written by the other author. Simulated data incorporated MP, TV, or a combination of both growth processes. The parameter values chosen (Table 2) were derived from previously published estimates for perch populations (Archibald et al. 1981; Leaman 1987, 1988) and from our unpublished data. The values selected for Φ_i^2 were chosen to be symmetric. To minimize computing requirements, we restricted the dimensions of the observation vectors by generating observations only for 5-yr intervals of age and only 4 natal yr. Therefore, the 20 values of j shown in the table span an age range of 100 yr.

The simulated data differed from real observations in several ways. First, the number of simulated observations from each 5-yr age group was equal. We chose this unrealistic age distribution because a main intent, for the simulated data, was to verify the performance of the parameter estimation program and check for local maxima of $\mathcal{L}^*$. The second difference was that we simulated observations for a cohort throughout its lifespan, i.e. we generated data of the second level of growth information referred to previously. Finally, we assumed that ages were known without error.

The problem of simultaneously estimating the P_i 's and the parameters that describe the g_i distributions is known to be difficult. When there is substantial overlap among adjacent g_i distributions, many observations are required to obtain reasonable parameter estimates. Typically, about 500 observations are necessary for mixtures of normal distributions under these circumstances. For this reason, we used 500 observations per mixed sample (fish of a given age and natal year) when generating simulated data.

Values for the independent parameters of Table 1 were used as input data for simulation. For each natal year (Ω), we calculated the proportions of each growth type at age using (7a),

TABLE 2. Parameter values used to generate the simulated LAA data. The last two columns list the maximum number for the indices Ω and j . Since ages were generated only for every 5 yr and natal years were generated only for every 25 yr, $j = 20$ corresponds to an age of 100 yr. Set No. 1 describes MP growth, set No. 2 describes TV growth, and set Nos. 3 and 4 describe a combination of these growth types.

Set No.	Model	Φ_1^2	Φ_2^2	Φ_3^2	L	L_M	K	K_M	m	σ	L_T	K_T	N	Ω	j
1	MP	0.2	0.6	0.2	40	2.5	0.9	-0.03	0.01	2	0	0	40 000	4	20
2	TV	1	0	0	40	0	0.9	0	0	2	2	-0.02	40 000	4	20
3	C	0.2	0.6	0.2	40	2.5	0.9	-0.03	0.01	2	2	-0.002	40 000	4	20
4	C	0.2	0.6	0.2	42.5	2.5	0.9	-0.03	0.03	2	2	-0.002	40 000	4	20

we calculated the mean LAA for each growth type using (8b), and finally, we generated the distribution of lengths about each mean following (8a). A uniform random number generator (IMSL subroutine RNUM) was used in combination with the Box-Müller algorithm to generate the uniform age distribution and the normal length distribution. Repeating this process over natal years produced the simulated observations of the LAA of individuals from different cohorts, throughout their lives.

Examination of the Parameter Estimates

Two objectives guided our tests with the simulated data. First, we wanted to demonstrate that, given sufficient data, model identification would be unambiguous. Therefore, we generated data that conformed to the combined, MP, or TV model. We fit these data sets with the parameters of the combined model constrained to emulate either the combined, MP, TV, or von Bertalanffy model. Likelihood ratio tests on the results of these fits identified the model that provided the best description of the data.

The second objective was to examine the error in the parameter estimates. We did not conduct an exhaustive sensitivity analysis of the parameter estimates for the simulated data. Such analyses, for multiparameter models, seldom convey a clear impression of the relative influence of parameters, due to interactions. Instead, we searched systematically for a global maximum of $\mathcal{L}^*$ by estimation from different initial parameter guesses. The coefficient of variation (CV) of the resulting estimates was used as a measure of precision.

Results

Parameter Estimates Using Simulated Data

Four different simulated data sets (Table 2) were generated to demonstrate the process of model selection and to test the performance of the parameter estimation program. These sets correspond to MP growth, TV growth, and two combinations of both MP and TV growth. The data sets were fit assuming either that one of the above models or a standard von Bertalanffy model applied. The resulting maximum likelihood values were tested to determine if model ambiguity exists when ample data are available.

Significant computer resources are required to estimate parameters for the simulated data. Because of the large sample sizes (40 000), evaluation of (6a) is time consuming. In addition, the likelihood surfaces are usually sufficiently flat in the neighbourhood of the maximum that many iterations are required. Parma and Deriso (1990) found similar requirements for sample size and behaviour of the likelihood surface.

Multiple pattern (MP) growth

Tests 1–4 of Table 3 are fits of the combined, MP, TV, and von Bertalanffy model, respectively, to data generated with set

No. 1 (Table 2). These data follow the assumptions of the MP model. When the correct model is chosen for the fit (test 2) the maximum likelihood is the highest and the parameter estimates are very close to their correct values. Test 1 has resulted in a very similar fit because the estimates for the time-varying parameters in the combined model are quite small. Both the TV and VB fits give smaller maximum likelihood values.

The $\mathcal{L}^*$ values from tests 1–4 can be used to identify the most appropriate model by performing a series of likelihood ratio tests (Kendall and Stuart 1979, chap. 24). Figure 5 shows the hierarchical model classification diagram for these tests. The combined model is at the top of the hierarchy, since all the other models are subsets of this model. When such a relationship exists between two models, it is shown in the diagram by a line joining related models. To be related to a higher model, all of the variable parameters in the lower (simpler) model must also be variable in the higher model. Thus, the MP and TV models are not hierarchically related to each other, but both are related to the combined model. At the bottom of the diagram, the VB model is shown to be a subset of both the MP and TV models, and therefore it must also be a subset of the combined model.

The likelihood ratio test compares twice the difference in the maximum log likelihood values with the relevant value of chi square. For example, to decide between the VB and MP models, we compare twice the difference in $\mathcal{L}^*$ values from Table 3 with chi square with 6 degrees of freedom. Since each of the four comparisons shown in Fig. 5 involves either 2 or 6 degrees of freedom, we need only the two reference values $\chi^2_{0.01,2} = 9.2$ and $\chi^2_{0.01,6} = 16.8$.

Double lines connecting related models in Fig. 5 indicate a significant improvement in describing the data by the more complex model. Thus, the MP model provides a significant improvement compared with the VB model, while the combined model provides a significant improvement compared with the TV model. The difference between the TV and combined models is just the unique components of the MP model. Since there was no significant improvement going from the MP to the combined model, the conclusion is that the MP model provides the best description of these data. This example demonstrates that, with sufficient data, model selection among these four candidate models can be objective and rigorous.

Time varying (TV) growth

Tests 5–8 in Table 3 are the result of fitting the same four models as above to the simulated TV data. The TV data are similar to MP data when $m = 0$ (the various cohort growth types have the same mortality). Therefore, fitting of either the combined or MP model to these data result in very small estimates for m . In addition, the MP fit (test 6) has attempted to describe the four TV growth regimes by three MP growth types. Thus, the estimate for L_M attempts to span the observed difference in asymptotic lengths, i.e. 3 times 2.68 is 8.04, while the data were generated with 4 times 2.0 or 8. Likewise, the

TABLE 3. Parameter estimates for simulated data and their maximum likelihood values. A dash indicates that the parameter was fixed at a value of 0. C, combined model; MP, multiple pattern model; TV, time-varying model; VB, von Bertalanffy model.

Test	Model for data	Model for fit	$-\mathcal{L}^*$	Φ_1^2	Φ_2^2	Φ_3^2	L	L_M	K	K_M	m	σ	L_T	K_T
1	MP	C	96781.5	0.21	0.58	0.21	40.0	2.46	0.90	-0.03	0.015	2.01	0.002	2×10^{-5}
2	MP	MP	96780.8	0.22	0.58	0.21	40.0	2.49	0.90	-0.03	0.014	1.99	—	—
3	MP	TV	97698.6	1	—	—	41.5	—	0.86	—	—	2.78	0.0002	0.0001
4	MP	VB	97691.2	1	—	—	41.5	—	0.87	—	—	2.78	—	—
5	TV	C	84779.5	0.52	0.48	0.0005	40.0	0.29	0.90	-0.005	0.013	2.01	2.0	-0.02
6	TV	MP	103656.2	0.31	0.34	0.35	40.3	2.68	0.90	-0.03	0.0006	2.08	—	—
7	TV	TV	84777.2	1	—	—	40.0	—	0.90	—	—	2.01	2.0	-0.02
8	TV	VB	104800.4	1	—	—	43.0	—	0.87	—	—	3.32	—	—
9	C	C	95909.9	0.22	0.58	0.21	40.1	2.43	0.90	-0.03	0.015	2.02	2.0	-0.02
10	C	MP	108333.2	0.18	0.49	0.32	42.0	3.04	0.89	-0.04	0.009	2.64	—	—
11	C	TV	96671.4	1	—	—	41.5	—	0.87	—	—	2.71	2.0	-0.02
12	C	VB	109193.2	1	—	—	44.5	—	0.84	—	—	3.71	—	—

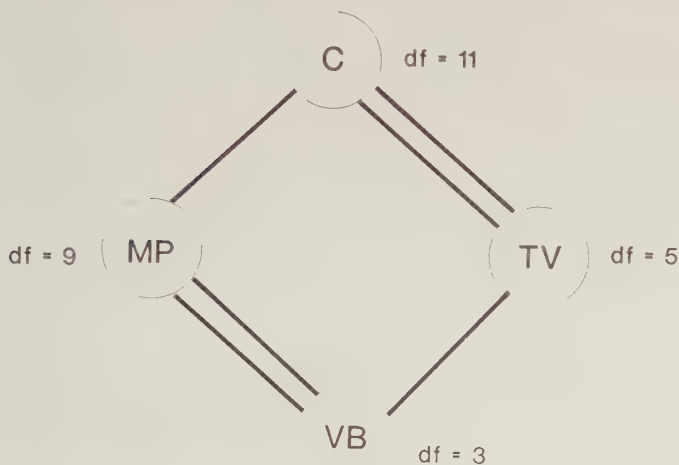


FIG. 5. Hierarchical model diagram for the MP simulated data. The most complex model is shown at the top of the diagram with simpler models shown below. Two models are joined by a single line if the lower one is a subset of the upper one. A double line signifies that a significant likelihood ratio test applies to the two related models. The conclusion drawn from this diagram is that the MP model provides the best description for these data. C, combined model; MP, multiple pattern model; TV, time varying model; VB, von Bertalanffy model.

estimate for K_M is the appropriate size, given that $G = 3$. In test 5, although $G = 3$, the estimates for Φ_3^2 , L_M , and K_M are such that essentially a $G = 1$ model has resulted. These two tests (5 and 6) demonstrate a universal problem with mixture models: determination of a unique value for G is very difficult.

Likelihood ratio tests were also carried out for these four fits. Both the MP and TV models provide significant improvements over the VB model, while the combined model is significantly better than the MP model. The explanation for the significant improvement of the MP fit over the VB fit is due to the similarity of the MP and TV models when $m = 0$, as described above. The additional improvement of the combined model over the MP model, and lack of significant improvement for the combined model as compared with the TV model, leads to the overall choice of the TV model. Once again, identification of the correct model is readily accomplished.

MP and TV growth

The majority of our investigations with simulated data were with data sets that followed the combined model assumptions.

Tests 9–12 in Table 3 give the results of fitting our four candidate models to data generated from set No. 3 (Table 2). With both growth type and time-varying effects present in these data, the likelihood for the correct model (test 9) shows quite an improvement over any other model. Likelihood ratio tests confirmed this. Every branch in the hierarchical model diagram for these tests showed that a significant improvement was obtained in going to the more complex model.

An additional series of parameter estimations was performed for the data generated from the combined model of set No. 4 (Table 2). Here, our objective was not model selection, but to learn something about the error in the parameter estimates. For these tests, we repeated the estimation procedure for a set of 16 initial parameter guesses, representing systematic departures from the correct values. These tests indicated only a single maximum of the likelihood, i.e. local maxima within the region of our initial parameter estimates seemed to be absent.

The final parameter estimates corresponding to different starting guesses do not converge to exactly the same values. Mixture problems are notorious for having a flat likelihood surface. There is always a region of the parameter space within which the estimates will not change substantially. For simplicity, we wrote a minimization program that used numerical approximations, rather than analytic expressions, for the partial derivatives of $\mathcal{L}^*$. These factors resulted in our obtaining slightly different sets of parameter estimates for each different set of initial guesses. We used these results simply to describe properties of the parameter estimation process.

We found, as with the tests of Table 3, that the TV growth processes are not confused with MP processes when adequate data are available. The parameter estimates for the combined model converged to near their true values (Fig. 6). However, the model parameters were not equally difficult to estimate (Fig. 6a). The low CV's for five parameters (σ , L , L_T , K , and K_T) show that they were well determined and that the model was robust to poor initial estimates. Another three (L_M , K_M , and m) were determined only moderately well, while Φ_1^2 , Φ_2^2 , and Φ_3^2 were difficult to determine with precision. The averages of the Φ_3^2 and m estimates showed the largest percentage bias; all others showed less than 2% bias from the true values (Fig. 6b). The proportion for the fastest growing component, Φ_3^2 , was consistently overestimated, while that for the slowest growing component, Φ_1^2 , was consistently underestimated. The major reason for the overestimation was the relatively short time over which this growth type was observed in LAA data.

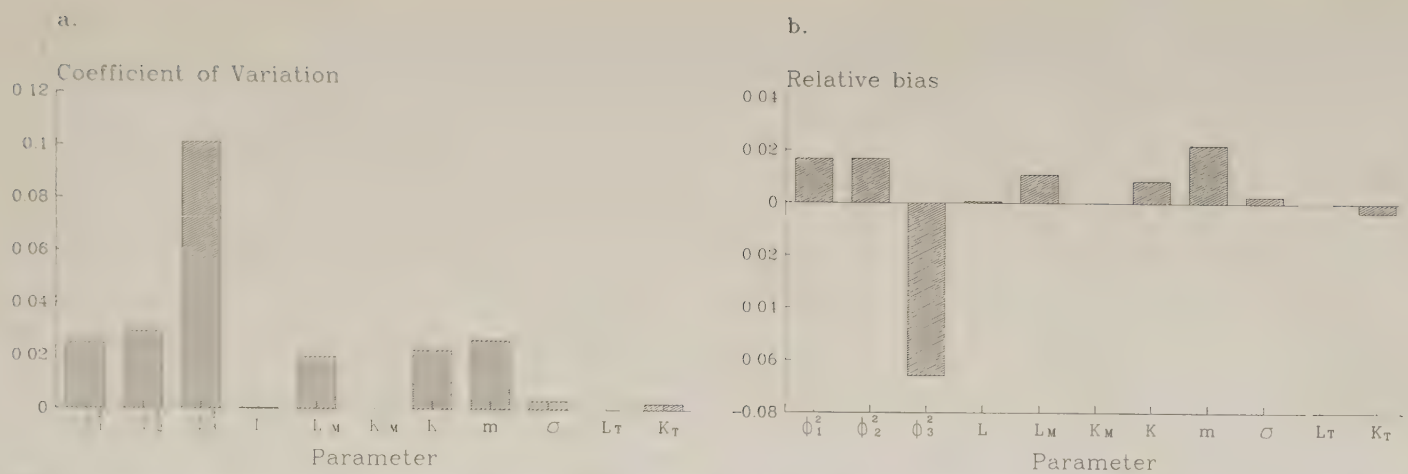


FIG. 6. Parameter estimation for the combined, MP, and TV simulated data set. (a) CV's for the parameter estimates. (b) Relative biases for the parameter estimates.

TABLE 4. Lower triangle of the correlation matrix for the parameter estimates for the combined, MP, and TV simulated data. Since the estimates for K and L_T did not vary, the correlation values for the corresponding rows and columns are left blank.

	Φ_1^2	Φ_2^2	Φ_3^2	L	L_M	K	K_M	m	σ	L_T	K_T
Φ_1^2	1.0										
Φ_2^2	0.940	1.0									
Φ_3^2	-0.960	-0.997	1.0								
L	0.336	0.607	-0.557	1.0							
L_M	0.976	0.985	-0.994	0.480	1.0						
K	—	—	—	—	—	1.0					
K_M	-0.946	-0.976	0.980	-0.507	-0.977	—	1.0				
m	0.452	0.714	-0.667	0.980	0.600	—	-0.614	1.0			
σ	-0.394	-0.110	0.171	0.653	-0.250	—	0.235	0.573	1.0		
L_T	—	—	—	—	—	—	—	—	—	1.0	
K_T	-0.448	-0.240	0.278	0.491	-0.350	—	0.312	0.376	0.851	—	1.0

TABLE 5. Final parameter estimates and their corresponding likelihood values for the perch data. A dash indicates that the parameter was fixed at a value of 0.

Test	Model fit	$-\mathcal{L}^*$	Φ_1^2	Φ_2^2	Φ_3^2	Φ_4^2	L	L_M	K	K_M	m	σ	L_T	K_T
1	MP	1507.2	0.99997	0.00003	—	—	44.8	1.47	0.85	-0.033	0.085	2.00	—	—
2	MP	1462.9	0.0003	0.9997	—	—	36.5	8.43	0.87	-0.023	0.086	1.93	—	—
3	MP	1492.5	0.0356	0.906	0.059	—	43.1	1.78	0.88	-0.034	0.027	1.79	—	—
4	MP	1452.9	0.00023	1.5×10^{-7}	0.052	0.948	35.9	3.14	0.88	-0.012	0.030	1.63	—	—
5	TV	1507.0	1	—	—	—	40.7	—	0.88	—	—	2.10	0.093	—
6	TV	1700.7	1	—	—	—	43.1	—	1.00	—	—	2.80	—	-0.00994
7	TV	1493.3	1	—	—	—	41.9	—	0.95	—	—	2.08	0.069	-0.00114
8	C	1452.6	0.00035	0	0.055	0.945	35.6	3.06	0.91	-0.012	0.027	1.64	0.013	-3×10^{-4}
9	MP	1452.9	0.00023	0	0.052	0.948	35.8	3.16	0.88	-0.011	0.030	1.63	—	—
10	TV	1496.8	1	—	—	—	42.7	—	0.94	—	—	2.03	0.056	-9×10^{-4}
11	VB	1507.2	1	—	—	—	44.8	—	0.85	—	—	2.00	—	—

This growth type has the highest mortality rate and, thus, is rapidly eliminated from the population.

Correlations among some parameter estimates were strong (Table 4), to be expected from the formulation of the model. The Φ_i 's were highly correlated due to the specific constraints of (6b). The parameters of incremental growth among the growth types (L_M and K_M) also showed high correlation ($r > 0.9$) with each other and with the Φ_i 's. The time-varying parameters (L_T and K_T) were extremely well determined and showed little correlation with other parameters, with the exception that K_T was positively correlated with σ ($r = 0.85$). Finally, m and L were also strongly correlated.

Parameter Estimates for Pacific Ocean Perch

We performed 11 different fitting tests (Table 5) with the perch data. Tests 1–4 assume an MP model with two, three, or four growth types. Comparison of tests 1 and 2 shows that with the same number of growth types (two), the model can lead to different parameter estimates. These two tests started with quite different initial parameter guesses. Thus, unlike the simulated data, the perch data do exhibit local maxima of the likelihood surface. Tests 5–7 assume a TV growth model with time dependence of L , K , or both. Tests 8–11 start with the param-

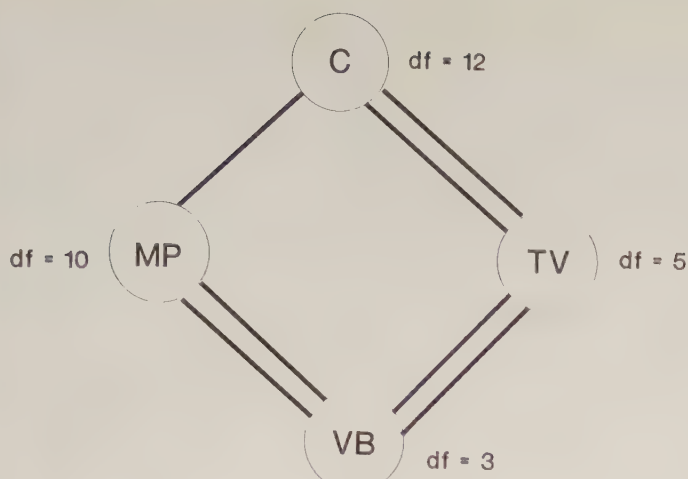


FIG. 7. Hierarchical model diagram for the Pacific ocean perch data. The diagram demonstrates that the MP model provides the best description of the four candidate models for the Pacific ocean perch data. The labels are the same as those of Fig. 5.

eter estimates from test 4 as initial guesses and enable model selection via likelihood ratio tests.

Tests 1 and 2 assume $G = 2$, while tests 3 and 4 assume G to be 3 and 4, respectively. For tests 2 and 4, the Φ_i^2 's result in a LAA distribution that is highly skewed towards small lengths. A model with $G = 3$ and a nonlinear dependence among the L_i 's, K_i 's, and m_i 's would give essentially identical results as test 4. The high $\mathcal{L}^*$ of test 4 is due to the inclusion of growth type $i = 1$ with a small asymptotic length (35.9). Test 2, which also includes a small asymptotic length for $i = 1$, yields the second highest maximum likelihood. The presence of this growth component accounts for the observations of small lengths at older ages (the "outliers" from the perspective of a standard, single growth type model), which are characteristic of these data (Fig. 1).

Given the small sample size for the perch data and the uncertainty in the Φ_i^2 estimates (compared with the simulated data), we take the Φ_i^2 estimates (Table 5) as indicative, rather than quantitative. Some of these estimates are quite small. One might wonder how a growth type with a proportion of 0.00023 (Φ_1^2 for test 4) can play any role in the fit. However, these parameters represent the proportion of the population at age 1. As the fish age, the relative contribution of this growth type increases as described by (7a). For example, at age 40 the relative proportion for this growth type is about 1%, while at age 80, it represents about 17% of the cohort survivors. The contribution of the slow-growing, long-lived fish is much more likely to be observed for the older fish in a sample, as is the case in Fig. 1.

The estimates for L_M , K_M , and m from tests 1–4 had the appropriate linkage with respect to the assumptions of the MP model. Specifically, all of these fits resulted in positive estimates for L_M , negative estimates for K_M , and positive estimates for m . We did not explicitly link these parameters mathematically, so that we would have the ability to determine if faster growing fish would be associated with larger asymptotic length and a shorter lifespan. The results reinforced our conviction that this type of linkage might be a common, naturally occurring phenomenon.

None of the fits of the TV model to the perch data resulted in as high a likelihood as the best of the MP model fits. We examined a time-dependent asymptotic length (test 5), a time-dependent growth rate (test 6), and a combination of both of

these time-dependent features (test 7). There was great disparity in the associated maximum likelihood values for these three fits. Test 7 had a likelihood value similar to MP growth with $G = 3$ (test 3), while test 5 was similar to MP growth with $G = 2$ (test 1).

Tests 8–11 provide the basis for model selection using likelihood ratio tests. Unlike the simulated data, the perch LAA data do not appear to follow any of our four candidate models exactly. In addition, the small number of observations (694) and uneven representation of the age categories result in parameter estimates with much larger errors than do the simulated data. Those caveats aside, the likelihood ratio tests provide a clear choice for the most appropriate model (Fig. 7). We used the parameter estimates of test 4 as the initial parameter guesses for these fits because this test provides the best overall likelihood value of all our fits. Thus, $G = 4$ for both C and MP models and we use the reference value $\chi^2_{0.01,7} = 18.5$ for significance testing. The likelihood ratio tests demonstrate that both the MP and TV models provide an improved description when compared with the standard VB model, as we suggested in the introduction. We see also that the combined model provides a significantly better fit than the TV model, but not better than the MP model. Therefore, we conclude from all four tests that the MP model provides the best description of the perch LAA data.

Discussion

Data Analyses

Our analyses of the perch and simulated data demonstrate that asymptotic growth of individual fish can lead to a LAA distribution in which older fish are smaller than younger fish. Indeed, more than one asymptotic growth model (MP or TV growth) can give almost identical LAA distributions. The likelihood ratio tests demonstrate that the perch LAA data are more likely to have come from an MP growth process than from a TV or VB process. Figure 8a shows explicitly how the MP growth process leads to smaller mean lengths for older fish. For this figure, we have calculated the mean length for each age by weighting the appropriate L_{i0} 's by their associated P_{ij} 's. Figure 8b shows that the difference between a von Bertalanffy growth function and a population governed by MP growth will exhibit the same systematic trend that we observed for the perch data (Fig. 2).

We used the simulated data to demonstrate certain points about model selection and parameter estimation, not to offer a specific alternative to the perch data. If LAA data are not available for several, sufficiently separated observation times, ambiguity between MP and TV growth may result. Conversely, when observations over several time periods and natal years are available, model ambiguity disappears and reasonable parameter estimates can be obtained. Clearly, LAA observations at one point in time are poor sources of information about the growth of individual fish, especially for species with longevity.

Our MP growth model differed from the multiple growth type model of Parma and Deriso in several ways. We chose a mathematically simpler, discrete model as opposed to their continuous model. The advantage of the discrete approach lies in its flexibility. Just as any continuous distribution can be approximated by an appropriate multinomial distribution, so the MP model can be made to approximate any desired length distribution. The linear relationships of the growth parameters (Table 1) were chosen for their simplicity. More complex

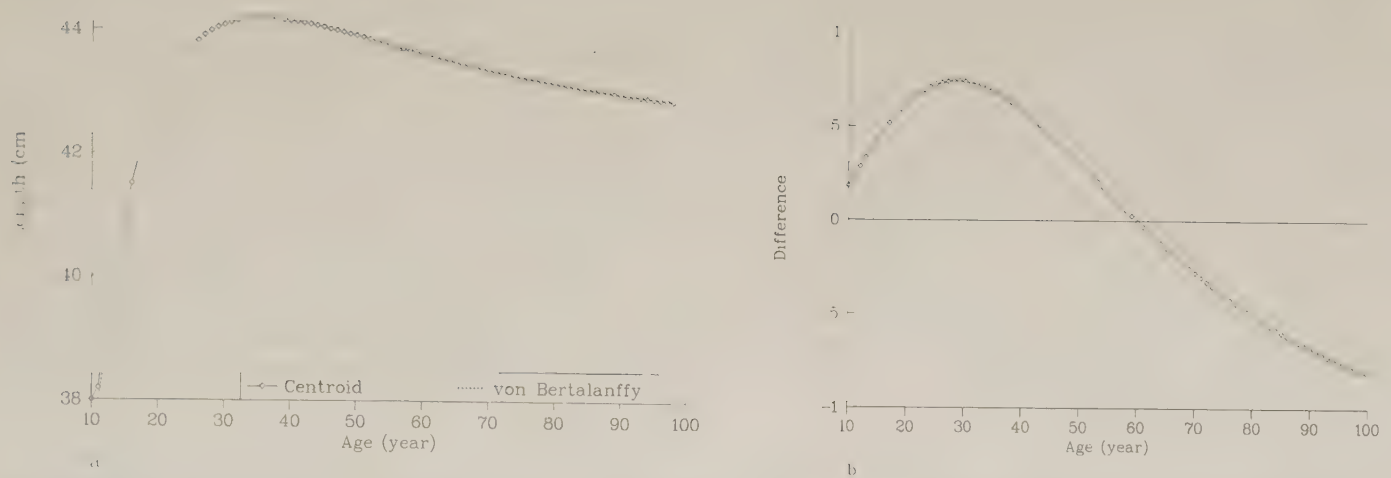


FIG. 8. Relationship between von Bertalanffy type asymptotic growth and MP growth. (a) Curves representing length centroid at each age for MP growth and mean length for von Bertalanffy growth. (b) Difference between the MP growth centroids and the von Bertalanffy lengths from Fig. 8a.

functional forms can be readily incorporated into the MP model with a minimum of additional parameters. Our focal point in developing this model was to understand the biological processes governing growth. This is reflected in our choice of a growth type dependent mortality. Parma and Deriso have concentrated on a better understanding of the fishery's influence on a population. Their choice of a size-dependent mortality is governed by this fact. These two approaches are complementary, rather than exclusive, models of reality.

Biological Implications

The initial observation which drew our attention to this problem was the occurrence of smaller, old fish and the fact that no asymptotic, single growth type model gave a satisfactory distribution of residual error. These phenomena suggested the possibility of different growth processes within the population. The results we have presented are encouraging, biologically. We demonstrated that the form of growth can be conservative, i.e. a general asymptotic function to describe the growth of individual fish, but still yield distributions which appear as if several growth functions are involved. We say conservative because the maintenance of separate growth functions is expensive, in an evolutionary or genetic sense.

Theilacker (1987) and Boehlert and Yoklavich (1984) have outlined physiological mechanisms for the MP growth process we describe. Theilacker showed that there are two types of larval clupeoids. Individuals may have either high growth rate or high conversion efficiency, but not both. Boehlert and Yoklavich demonstrated that conversion efficiency varied inversely with ingestion rate. If these mechanisms are general, then the smaller, older perch may be optimizing conversion efficiency, rather than growth. Westheim's (1973) observation of smaller LAA for perch in deeper water, where there is presumably less available energy, lends support for such a mechanism. We have taken these observations on conversion efficiency versus growth rate a step further by suggesting that mortality is intimately linked to these processes.

The correlation between growth rate and survival rate which leads to the MP growth process has attractive features which seem to unify some disparate observations. For this reason (as opposed simply to its success in describing LAA data), it

represents a theory of interest. For the population dynamicist, there are some important implications of our findings. If exploitation has truncated the age spectrum of a population, the estimated L_{∞} calculated from present LAA information may be overestimated because of the absence of these older, smaller fish. This may have subsequent effects on the perception of the surplus production for the population. It may also be interpreted incorrectly as a density-dependent growth response. If fishing gear is selective for faster growing fish at the younger ages, parameter estimation may be affected in the same way as by a lack of older fish in the population.

The effects of exploitation on animals such as these also have implications for understanding processes such as evolutionary persistence. A failure to observe and consider the importance of multiple growth types or growth-survival interactions may preclude the understanding of important mechanisms for evolutionary success. Consideration of the selective pressures which have given rise to this combination of growth and longevity (e.g. uncertain reproductive success) will help focus attention on the characteristics of populations which may have to be conserved to ensure both biological and economic persistence.

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We wish to acknowledge the encouragement of Jon Schnute to undertake this problem and to thank him for his helpful suggestions about improvements to our model. Thanks are also due to Rob Kronlund for reading the manuscript and providing many useful suggestions for its improvement. Thoughtful comments by the three referees encouraged us to develop new aspects of the model and provided considerable improvement to our presentation.

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Relationship between Food, Fat, Sexual Maturation, and Spawning Time of Baltic Herring (*Clupea harengus membras*) in the Archipelago Sea

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The effect of nutritional status on gonad maturation and timing of spawning was examined in the Baltic herring (*Clupea harengus membras*) in the Archipelago Sea. Fish were collected from overwintering areas in December and from the spawning grounds in May–July. Muscle fat content, amount of mesenteric fat, and condition factor were used as indicators of nutritional status of fish. In winter, fish were highly variable with substantial individual variation in nutritional status, gonad stage, gonad weight, and gonadosomatic index (GSI). Gonad weight was related to fat content, suggesting a close relationship with fish nutritional status and maturation rate. Spawning fish were separable into early and late spawners according to fat content, gonad weight, and GSI but not according to length. The spawning shoals consisted of mixtures of fish of all sizes. I concluded that in the study area, individual maturation cycles vary and timing of spawning is primarily determined by the feeding conditions prior to spawning.

Les effets de l'état nutritionnel sur la maturation des gonades et le moment du frai ont été étudiés chez le hareng baltique (*Clupea harengus membras*) de l'archipel Arctique. On a recueilli des poissons dans des zones d'hivernage en décembre et des frayères en mai–juillet. On a utilisé la teneur en lipides des muscles, la quantité de lipides mésentériques et la condition des poissons comme indicateurs de l'état nutritionnel du poisson. En hiver, les données sur les poissons étaient très variables et présentaient des variations individuelles importantes pour ce qui est de l'état nutritionnel, du développement et du poids des gonades, et de l'index gonadosomatique (IGS). On a établi une relation entre le poids des gonades et la teneur en lipides, qui suggérait une bonne corrélation entre l'état nutritionnel du poisson et le degré de maturation. On pouvait classer les géniteurs en géniteurs de début et de fin de saison selon la teneur en lipides, le poids des gonades et l'IGS mais non selon la longueur. Les frayères comportaient des mélanges de poissons de toutes les tailles. J'ai conclu que dans le secteur à l'étude, les cycles individuels de maturation varient et le moment du frai est déterminé d'abord par les conditions d'alimentation avant le frai.

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Herring spawning times have often been thought to be genetically fixed (see Smith and Jamieson 1986). Based on this assumption, Baltic herring (*Clupea harengus membras*) has been divided into two groups according to spawning time: spring-spawners and autumn-spawners. Recently, Aneer (1985) criticized this division because the two groups may not be genetically different (Ryman et al. 1984). In the Finnish southwestern archipelago, most herring are "spring-spawners." Total annual catch is about 24 000 t, of which about 25% is caught with trap nets during the spawning season (FGFRI, Fisheries Division 1989). Peak spawning takes place from May to July in the innermost parts of the archipelago (Rajasilta 1982), but in some years, spawning may be continuous from the middle of April to the middle of August in the same area (M. Rajasilta et al. unpubl. data). Therefore, division of the stock into spring- and autumn-spawners is not correct in this case, although fish spawning in August are usually called autumn-spawners. Instead of separating herring into two spawning groups, the reproductive pattern of herring can be better described as a temporal continuum, where fish mature and spawn over a long time span (see also Smith and Jamieson 1986). The spawning times reported around the Baltic Sea show that a reproductive continuum is typical of herring (Rannak

1959; Hahtonen and Joensuu 1984; Klinkhardt et al. 1985; Oulasvirta et al. 1985), but the biological basis of the phenomenon is unknown.

Continuous recruitment of fish into the spawning population means that maturation within the stock is asynchronous. However, precise timing of spawning of individual fish within the continuum is difficult to explain on a genetical basis. Maturation of Pacific herring (*Clupea harengus pallasi*) is temperature dependent (Ware and Tanasichuk 1989), and also, feeding influences maturation rates (Hay and Brett 1988). Obviously, regulation of spawning time is under ecological influence both from the physical and biological components of the environment, e.g. feeding conditions prior to spawning may affect the maturation of fish and thus determine timing of spawning. Aneer (1985) claimed that spawning time of Baltic herring is dependent on nutritional status: fish that accumulate all the energy necessary for the next spawning already in winter spawn in spring. Those who do not accumulate sufficient energy spawn later when they have completed their energy storage during the early spring and summer.

This paper analyses maturation processes of Baltic herring. The relationship between fish nutritional status and gonad stage

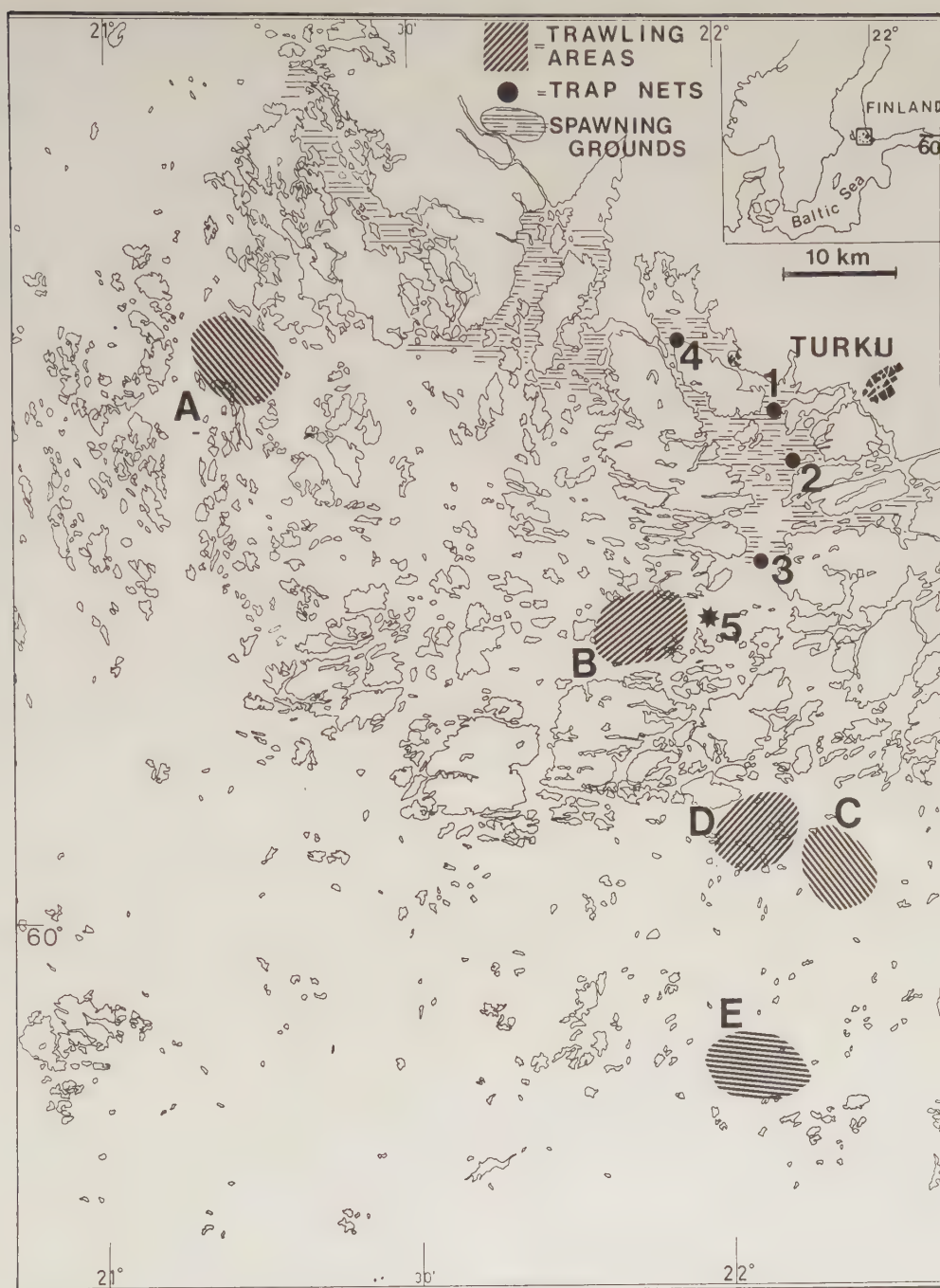


FIG. 1. The Archipelago Sea and sampling sites (A–E = locations of winter sampling; 1, 2, 3, and 4 = trap net sampling on the spawning grounds; 5 = gillnet sampling).

was studied to find the role of feeding conditions in the regulation of maturation processes and spawning time.

Material and Methods

Study Area

The Archipelago Sea off the southwest coast of Finland (Fig. 1) is one of the most extensive archipelagos in the world. Due to the complex morphology, it forms a heterogeneous and diverse environment, which is different from most other coastal areas in the Baltic Sea. The area is shallow (mean depth about 20 m) and salinity and temperature conditions are different from

the open sea (Mälkki and Tamsalu 1985). Ice cover usually lasts 3–3.5 mo in the inner parts of the area, the formation of the permanent ice beginning in early January (Kalliosaari and Seinä 1987). Mean temperature of the seawater varies between 0 and 19°C at the surface (maximum in August) and between 0 and 9°C at a depth of 40 m (maximum in October) during a year (statistics from the years 1967–84; Archipelago Research Station). During winter, the water column lacks thermal stratification. The salinity of the surface water is 6–7‰, and because of the shallowness, no halocline is formed.

Fish Sampling and Treatment

Spawning fish were collected in 1987 and 1988 (Fig. 2) from commercially used trap nets which were located on known

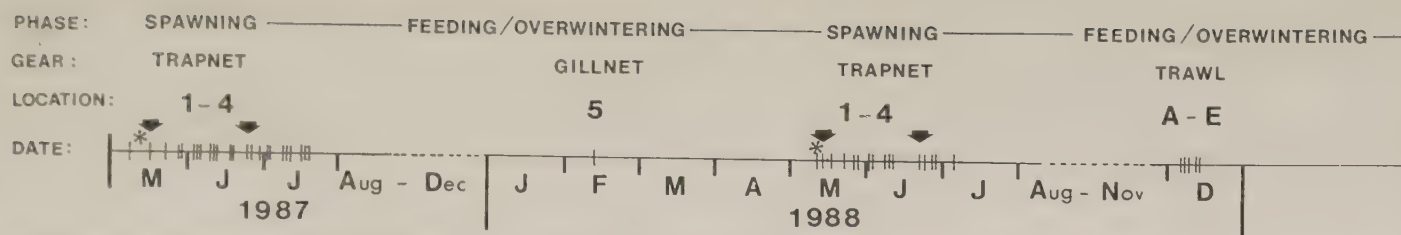


FIG. 2. Sampling schedule for herring in different phases (spawning or feeding/overwintering) in 1987 and 1988. Asterisks indicate the appearance of first spawn in the spawning area, vertical bars indicate the sampling dates, and arrows indicate the dates when fish for comparison of the early and late spawners were taken from trap nets.

spawning shores (study locations 1–4, Fig. 1). The seabed in the vicinity of the trap nets was monitored by divers during the main spawning period (April/May–July). Fish sampling was started when eggs first appeared on the bottom. Trap net samples were taken continuously at 1- to 10-d intervals until spawning was over. Before emptying the trap net, fish were led into one corner of the trap by lifting the bottom net. Random samples of 100–150 individuals were taken into the laboratory. Total length (in millimetres from the tip of the snout to end of the tail fin) and weight (0.1 g precision) of fish were measured and sex was determined. Otoliths were taken for age determination and the developmental stage of the gonads was estimated using a classification simplified from Kesteven (1960). The following developmental stages were identified: stage 1, undeveloped gonads, sex usually unidentifiable; stage 2, gonads about one third of the body cavity; stage 3, gonads one third to two thirds of the body cavity; stage 4/5, gonads greater than two thirds of the body cavity and maturing; stage 6, gametes running.

In 1988 the total gonad weight was measured to the nearest 0.01 g in all fish. Fish for fat analysis were wrapped individually in aluminium foil, packed tightly in plastic bags to avoid desiccation, and stored at -20°C until analysis.

Winter fish samples were collected within 1 wk (December 7–13, 1988) from commercial trawls at five locations in the Archipelago Sea (trawling areas A–E, Fig. 1). A random sample of 100–150 fish was treated in the same way as the trap net samples except that age determinations were not made. An additional sample for fat analysis of young fish was collected with gill nets in February 1988 at location 5 close to the spawning grounds (Fig. 1).

Age was determined from the otoliths in all trap net fish (data not presented in this paper), and 2-yr-old fish were separated from the rest of the material after otolith readings. From the trawl fish, this age group was selected using age–length keys calculated from the trap net material.

According to age determinations made in 1987 and 1988, length of 2-yr-old fish varied between 12.0 and 15.9 cm ($N = 49$) and that of 3-yr-old fish between 15.2 and 19.2 cm ($N = 50$). Thus, all fish between 12.0 and 15.0 cm in the trawl samples were taken as 2-yr-old potential recruit spawners.

The amount of fat deposited around the gut of fish was estimated on a relative scale from 1 to 5 (1 = no fat; 2 = fat distinguishable as a narrow white thread along the gut; 3–5 = increasing fat deposits around the gut and stomach). The stomachs and contents were preserved in a 70% ethanol solution. The stomach fullness was estimated on a relative scale from 1 to five (1 = empty; 5 = full) and stomach contents were identified. Condition factor was calculated using Fulton's condition factor (K) = $100 \times \text{weight}/\text{length}^3$ (Bagenal and Tesch 1978; see also Parmanne 1990). Gonadosomatic index (GSI)

was expressed as the ratio of gonad fresh weight to total fresh weight of fish (with gonads and intestines).

Fat Analyses

Fat analyses followed the procedures by Linko et al. (1974). A piece of dorsal muscle (3–4 g fresh weight), taken between the head and dorsal fin, was homogenized and mixed in a mortar with 6–8 times the amount (grams) of anhydrous sodium sulphate. The homogenate was dried overnight and extracted for 6 h with diethyl ether in a Soxhlet apparatus. The solvent was evaporated and the fatty residue weighed (0.1 mg precision). The analyses were made on individual specimens from samples each consisting of about 20 fish. As the fat content did not differ according to sex (Mann–Whitney $U = 312$; $p > 0.10$) (Table 1), the sexes were combined for comparison of areal differences in winter. However, males were mostly treated as pooled samples: from 15–20 fish of different size (14–24 cm), a similar piece (1.00 g) of the dorsal muscle was taken and homogenized. The homogenate (3–4 g) was analysed as above. For the comparison of early and late spawners, only females were used.

Statistical Analyses

The data were analysed using the Statview statistical package (Feldman and Gagnon 1986) and SAS/STAT software package for personal computers (SAS Institute Inc. 1988). Before the analyses, possible deviations from normality in the distributions of the parameters were examined and if needed, transformations ($\log(x)$, square root, or arcsin square root) were used to reduce nonnormality. When using one-way ANOVA, linear correlation, or regression analysis, transformed data were used in some cases (given in text, tables, or figures). If the transformations were not effective enough or if ordinal data were used, nonparametric tests (Mann–Whitney U -test, Kruskal–Wallis, and Spearman rank correlation (r_s)) were employed.

Results

Winter Samples

Herring collected in December 1988 from trawl catches were mainly 14–18 cm long (mean about 16 cm in both males and females in all study locations; Table 1). Juveniles (small fish with undeveloped sexual organs) were present in all samples but in varying numbers. The highest proportion (39% of the total sample) was observed at location B, which is situated close to the spawning grounds (see Fig. 1).

The size distributions of males and females were similar (one-way ANOVA; $p > 0.10$ in both sexes) in all sampling areas (Table 1). Gonad weight and GSI also were similar throughout the areas, but the condition factor of females varied ($p < 0.05$); in area B, it was somewhat lower than in areas A and D.

TABLE 1. Fish length, condition factor, gonad weight,^a gonadosomatic index (GSI),^a and fat content of dorsal muscle (mean, standard deviation, and range) of the trawl fish from areas A–E in December 1988 (note different *N* of fish in fat analyses). At the bottom of the table is a comparison of different variables between the areas A–E with one-way ANOVA (length, condition factor, gonad weight, and GSI) or with the Kruskal–Wallis *H*-test (fat content).

Area	Date	Sex		Length (cm)	Condition factor	Gonad wt (g)	GSI	Fat content (%)	<i>N</i>
A	Dec. 13	Males (<i>N</i> = 54)	Mean (SD)	16.0 (2.0)	0.69 (0.07)	3.26 (3.48)	0.088 (0.053)	10.2 (5.8)	10 (8.9 pooled sample)
			Range	12.7–25.8	0.56–0.86	0–22.2	0–0.190	3.3–19.9	
		Females (<i>N</i> = 38)	Mean (SD)	16.8 (2.0)	0.68 (0.06)	1.59 (1.41)	0.042 (0.028)	6.9 (3.1)	18
			Range	13.0–22.3	0.59–0.84	0–5.2	0–0.119	2.7–15.0	
		Juveniles (<i>N</i> = 11)	Mean (SD)	12.8 (1.9)	0.62 (0.04)	—	—	—	
			Range	8.7–15.2	0.53–0.67	—	—	—	
B	Dec. 7	Males (<i>N</i> = 46)	Mean (SD)	16.2 (2.0)	0.68 (0.08)	3.38 (2.58)	0.099 (0.048)	—	14
			Range	13.2–22.6	0.55–1.07	0–11.4	0–0.177	—	
		Females (<i>N</i> = 29)	Mean (SD)	15.9 (2.8)	0.65 (0.05)	1.72 (2.49)	0.042 (0.037)	7.2 (2.8)	14
			Range	13.2–25.5	0.56–0.73	0–10.0	0–0.125	4.0–13.4	
		Juveniles (<i>N</i> = 49)	Mean (SD)	10.3 (2.3)	0.56 (0.06)	—	—	—	
			Range	7.3–14.4	0.38–0.66	—	—	—	
C	Dec. 7	Males (<i>N</i> = 36)	Mean (SD)	16.3 (2.8)	0.68 (0.05)	3.65 (5.13)	0.080 (0.062)	—	
			Range	12.6–25.4	0.60–0.84	0–23.0	0–0.224	—	
		Females (<i>N</i> = 47)	Mean (SD)	16.4 (2.2)	0.66 (0.06)	2.21 (2.76)	0.055 (0.044)	—	
			Range	13.0–22.0	0.40–0.75	0–10.9	0–0.161	—	
		Juveniles (<i>N</i> = 19)	Mean (SD)	13.6 (0.9)	0.62 (0.03)	—	—	—	
			Range	10.5–14.7	0.58–0.73	—	—	—	
D	Dec. 8	Males (<i>N</i> = 47)	Mean (SD)	16.6 (2.3)	0.67 (0.06)	3.71 (3.47)	0.092 (0.057)	—	20
			Range	13.5–22.2	0.52–0.85	0–12.2	0–0.186	—	
		Females (<i>N</i> = 38)	Mean (SD)	16.8 (2.6)	0.67 (0.04)	2.27 (2.68)	0.049 (0.040)	8.9 (3.6)	20
			Range	13.0–24.7	0.58–0.74	0–11.8	0–0.144	3.6–17.1	
		Juveniles (<i>N</i> = 15)	Mean (SD)	12.7 (2.2)	0.61 (0.04)	—	—	—	
			Range	7.8–15.0	0.55–0.67	—	—	—	
E	Dec. 13	Males (<i>N</i> = 51)	Mean (SD)	17.0 (2.0)	0.66 (0.05)	3.25 (2.81)	0.083 (0.053)	6.3	Pooled sample
			Range	13.8–21.2	0.58–0.77	0–10.4	0–0.164	—	
		Females (<i>N</i> = 30)	Mean (SD)	16.9 (2.4)	0.67 (0.05)	1.63 (1.51)	0.040 (0.025)	7.2 (4.1)	20
			Range	13.3–22.3	0.59–0.79	0–6.13	0–0.091	3.3–16.3	
		Juveniles (<i>N</i> = 30)	Mean (SD)	13.5 (1.5)	0.62 (0.05)	—	—	—	
			Range	9.0–15.7	0.52–0.82	—	—	—	
ANOVA		Males	<i>F</i>	1.386	2.180	0.214 ^b	0.632	Kruskall–Wallis <i>H</i> = 4.113, <i>p</i> > 0.10	
			df	4, 225	4, 225	4, 223	4, 223		
			<i>p</i>	0.239	0.072	0.930	0.640		
		Females	<i>F</i>	1.147	2.516	0.203 ^c	0.200 ^c		
			df	4,191	4, 191	4, 180	4, 180		
			<i>p</i>	0.336	0.043	0.936	0.938		

^aGonad weights <0.01 g and GSI's <0.001 are given as 0.

^bData square root transformed prior to analysis.

^cData log(*x*) transformed prior to analysis.

Most fish had reached maturation stage 3 in December (Fig. 3A). The maturation stage was positively correlated with the size of the fish (females: $r_s = 0.528$; $p < 0.001$; $N = 196$; males: $r_s = 0.478$; $p < 0.001$; $N = 230$), but the variation was high even in fish of the same size. In both sexes, well-developed gonads (stage 4/5 or 6) were found in fish of all size groups, but the percentage was higher in males (42%) than in females (17%). Some males were already ripe although spawning did not start until late April of the following spring (1989). The size distribution of fish with ripening or ripe gonads (stages 4/5 and 6) ranged from 13 to 25 cm in the samples (Fig. 3B) and the relationship of fish length to gonad weight or GSI was positive and significant (Fig. 4). However, individual variation was conspicuous in each sample (i.e. GSI ranged between 1 and 12% in females and between 1 and 14% in males of 16 cm total length).

Fat content of the muscle increased with size of the fish, but fish length accounted for only 20.8% of the variation

($p < 0.001$; both sexes combined; see Fig. 5A). Maximum values were found in fish of 18–20 cm length whereas the biggest fish seemed to have less fat in their muscle. Between the sampling areas, no differences existed ($p > 0.10$; Table 1).

Gonad weight increased with the increase of fat content of female muscle, but not in a linear way (Fig. 5B). After reaching a level of about 12–14%, the amount of fat increased without similar increase in gonad weight. Females with rich fat deposits in the muscle were all 18–21 cm long but their GSI values ranged from 3.1 to 10.9%. However, this did not differ significantly from GSI values in females of the same size but having fat less than 14% ($U = 66.5$; $p > 0.10$). Gonad maturation stages increased with increase of the fat content of dorsal muscle (Fig. 5C), but the amount of fat deposited around the gut was inversely correlated with gonad weight ($r_s = -0.526$; $p < 0.001$; $N = 497$; both sexes combined). Condition factor also indicated the fat content of the muscle; in females, a positive correlation was found between the two

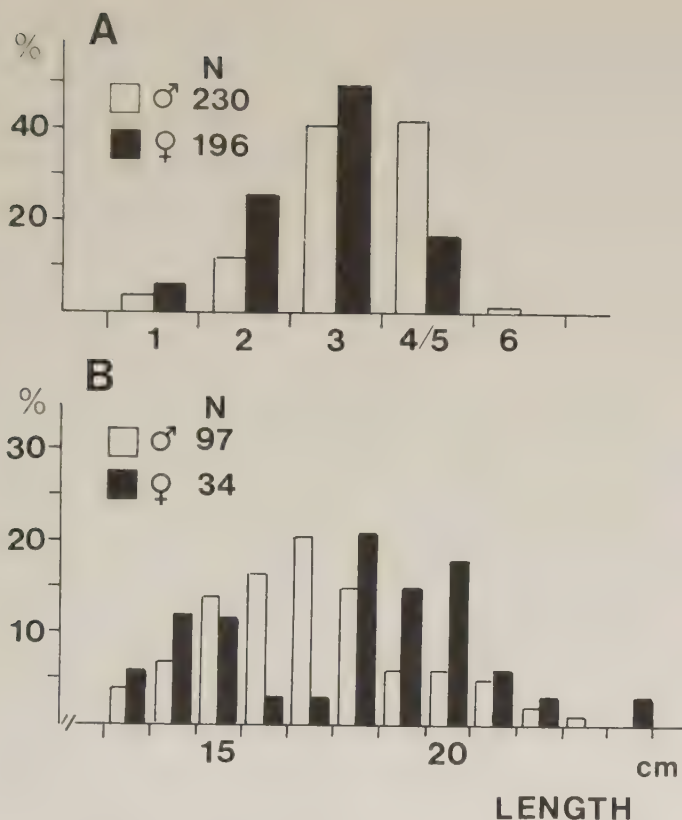


FIG. 3. (A) Frequencies of the gonad stages in herring collected in December 1988 in areas A-E. (B) Size distribution of males and females in gonad stages 4/5 and 6 in December 1988 in areas A-E.

parameters ($r = 0.569$; $p < 0.001$; $N = 87$). In the same way, condition factor correlated positively with gonad weight (females: $r = 0.549$; $p < 0.001$; $N = 185$; males: $r = 0.501$; $p < 0.001$; $N = 218$).

The stomach analyses ($N = 76$) showed that fish caught in December were still feeding. Only 12% of the stomachs were totally empty and most fish had at least some food remains, although the prey eaten was in many cases too digested to be identified. Interestingly, about 57% of the fish studied had eaten littoral amphipods (*Gammarus* spp.) instead of zooplankton and some individuals had eaten young herring. The type of food was the same in all sampling areas.

Spawning Fish

Fish taken from the trap nets during the spawning season in May-July were mainly of ages 3-5. Mean length varied between 1987 and 1988 (Fig. 6), but with no clear relationship to either spawning time or location. In 1987, spawning started in May at location 1, where the first shoal consisted of a high number of small-sized fish, the mean lengths of both males and females being < 17 cm. In 1987, the proportion of large individuals in the shoal sampled was highest in the beginning of June at location 2 as the sample mean length shows. In 1988, spawning started almost simultaneously at locations 1 and 2. Here, females were larger than those coming later but such a trend was not found in males. Differences in length between the samples were significant (one-way ANOVA) in both 1987 (males: $F = 6.45$; $p < 0.001$; $df = 23, 1177$; females: $F = 5.63$; $p < 0.001$; $df = 23, 1010$) and 1988 (males: $F = 2.28$; $p < 0.005$; $df = 15, 706$; females: $F = 4.31$; $p < 0.001$; $df = 15, 603$).

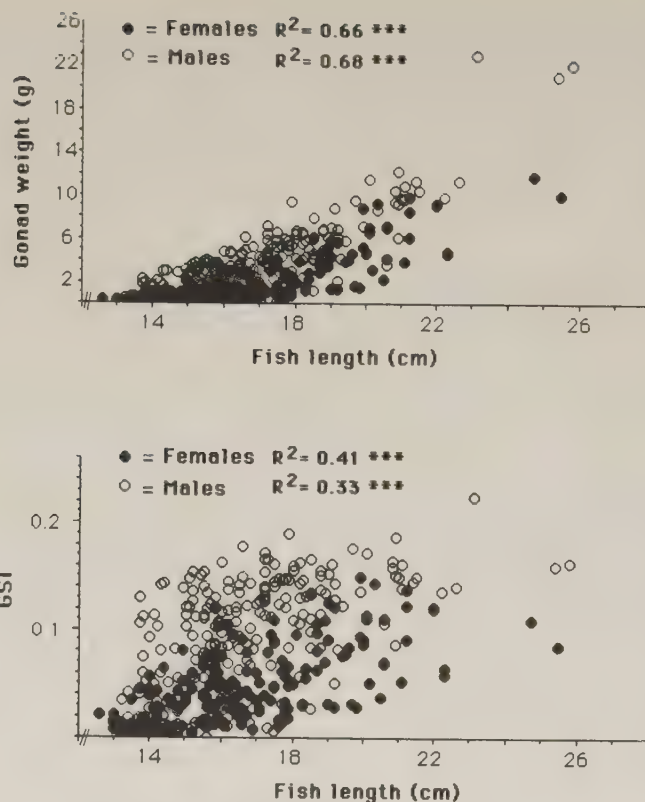


FIG. 4. Gonad fresh weight and gonadosomatic index (GSI) of males (gonad weights square root transformed prior to analysis) and females (gonad weights and GSI's log(x) transformed prior to analysis) in relation to fish length at study locations A-E in December 1988. ***Significant at $p < 0.001$.

In both the early and late spawners, some individuals were at the stage 3, although the number of these fish was low through the summer (mean of the trap net samples in May-July 1987 = 7%; $N = 1890$). In both sexes, some individuals had low gonad weight, although the gonad stage was 4/5 or 6 and fish were rather large (see Fig. 7). Gonad weights and GSI's of the early spawning fish were higher than those of the late spawners in both sexes. The shoals consisted of fish of different size: the difference existed even when the effect of length was eliminated and only fish of a given size range were compared (16.0-20.0 cm; differences in length between the groups insignificant; see Table 2). In the comparison, only fish in gonad stage 6 were considered. The condition factor of the early spawning males was higher than that of the late spawning males ($p < 0.01$) but equal in females irrespective of the spawning time ($p > 0.10$).

Fat content of females was lower in the late spawners (mean = 1.7%; years and locations combined) than in the early ones (mean = 5.4%; $U = 17.0$; $p < 0.01$). Variation was high in early reproducing females, some of which had lipid concentrations as high as 10-11% even when spawning (Fig. 8). In late reproducing females, however, the maximum values did not exceed 3.5% and the variation between individuals was small.

Fish at First Maturation

Sampling made during the spawning period at study sites 1-4 showed that a small number of maturing or ripe (gonad stages 4/5 and 6) 2-yr-old fish was present in almost all spawning shoals (Fig. 9). The proportion of fish reproducing at this

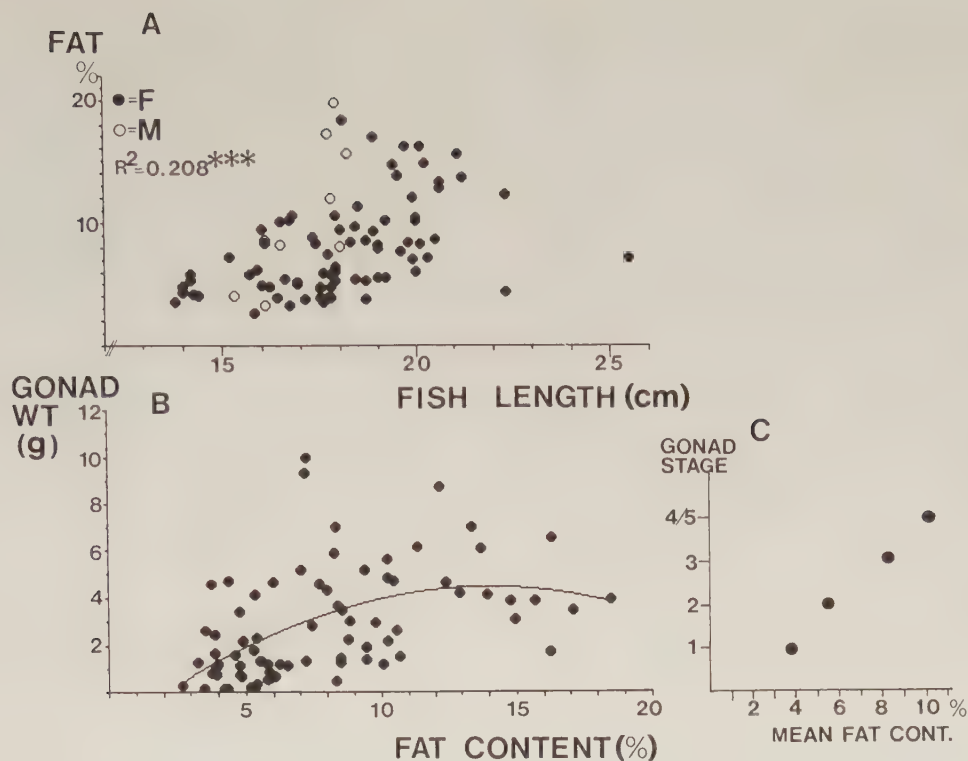


FIG. 5. (A) Fat content of the dorsal muscle (% fresh weight, arcsin transformed values used) in relation to fish length in December 1988 (sexes combined for R^2), (B) gonad weight of females in relation to muscle fat content (% fresh weight, arcsin transformed values used) in December 1988 (curve fitted by eye), and (C) gonad stage in relation to mean content of muscle fat (sexes combined). ***Significant at $p < 0.001$.

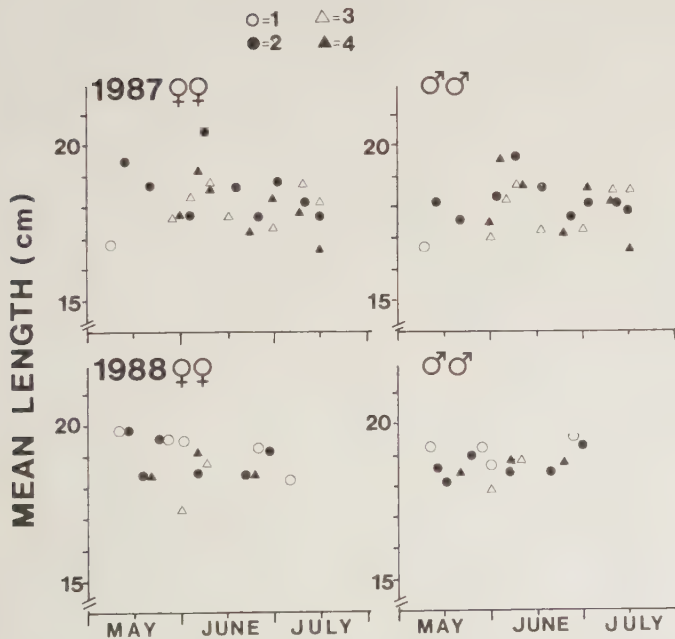


FIG. 6. Mean length of males and females at locations 1, 2, 3, and 4 during the spawning season in 1987 and 1988 (May–July).

age varied between 0 and 9% of all fish in the sample. Both sexes occurred in the shoals throughout the season. However, not all 2-yr-old fish were sexually mature, in spite of their presence in the spawning shoals. In every shoal, some individuals had gonads in early developmental stages (2 and 3), and in some juveniles, sex could not be determined.

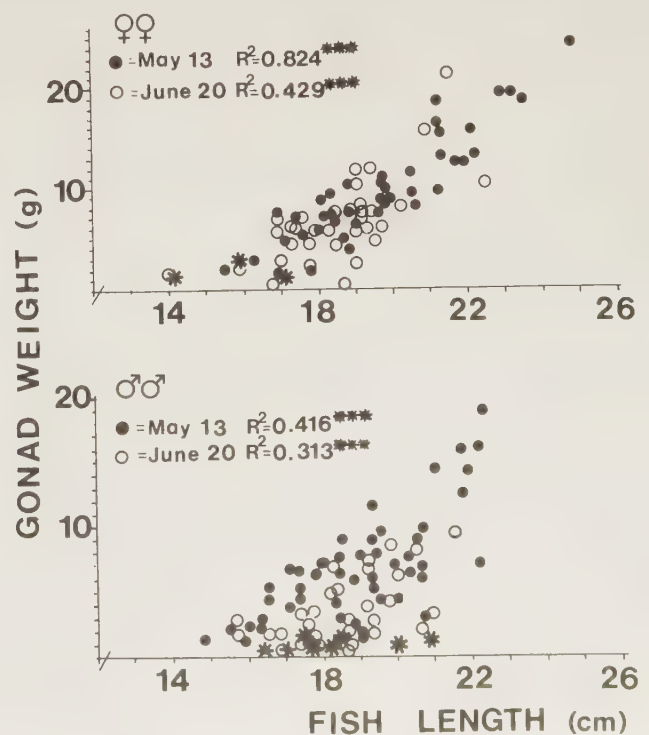


FIG. 7. Gonad fresh weight in relation to fish length in the first and last spawning waves in 1988 at location 2. Asterisks show fish in gonad stages < 4. ***Significant at $p < 0.001$.

TABLE 2. Comparison of early and late spawning herring (gonad stage 6) in 1988 (Mann-Whitney *U*-test). For further explanations, see text.

Sex	Variable	Early spawn	Late spawn	<i>U</i>	<i>p</i>
Males	Length				
	Mean	18.6	18.4	124.5	>0.10
	SD	1.0	1.0		
	Gonad wt				
	Mean	5.5	3.8	91.5	<0.05
	SD	2.8	2.2		
Females	GSI				
	Mean	0.13	0.09	94.0	<0.05
	SD	0.05	0.04		
	Condition factor				
	Mean	0.66	0.61	64.0	<0.01
	SD	0.06	0.05		

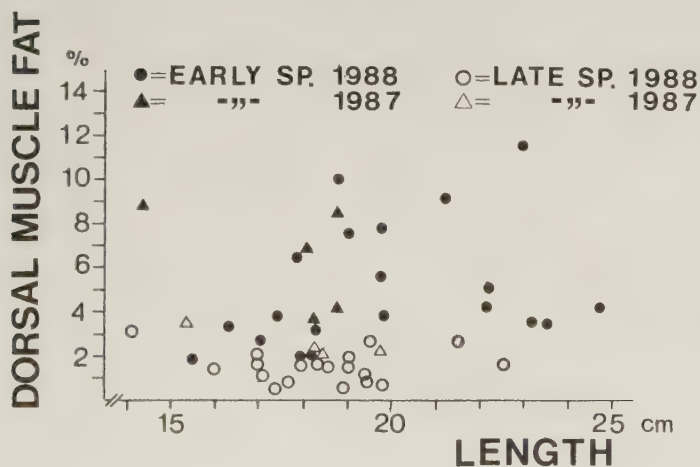


FIG. 8. Fat content of the dorsal muscle (% fresh weight) in relation to length in early (May) and late (June–July) spawning females in 1987 and 1988. Fish maturing or spawning = gonad stages 4/5 and 6.

The length of 2-yr-old maturing or spawning fish (gonad stages 4/5 and 6) varied from 13 to about 16 cm in both sexes (Table 3). Juvenile 2-yr-old fish present in the shoals did not differ in length from the males or females of the same age ($p > 0.10$; see Table 3), but in both sexes, condition factor was lower (males: $U = 25$; $p < 0.001$; females $U = 56$; $p < 0.001$). No difference was found between the spawning males and females ($U = 203$; $p > 0.10$). For this comparison, fish in the trap net samples were combined for the years (1987 and 1988), locations (1, 2, 3, and 4), and spawnings, due to a low number of 2-yr-old spawners in the shoals.

Of the age 2 fish in December, about 8% of females and 20% of males were at gonad stage 4/5 (Fig. 10A). The GSI of males reached as high as 14%, and in about 20% of males, it exceeded 10%. In females, maturation had not proceeded as far as in

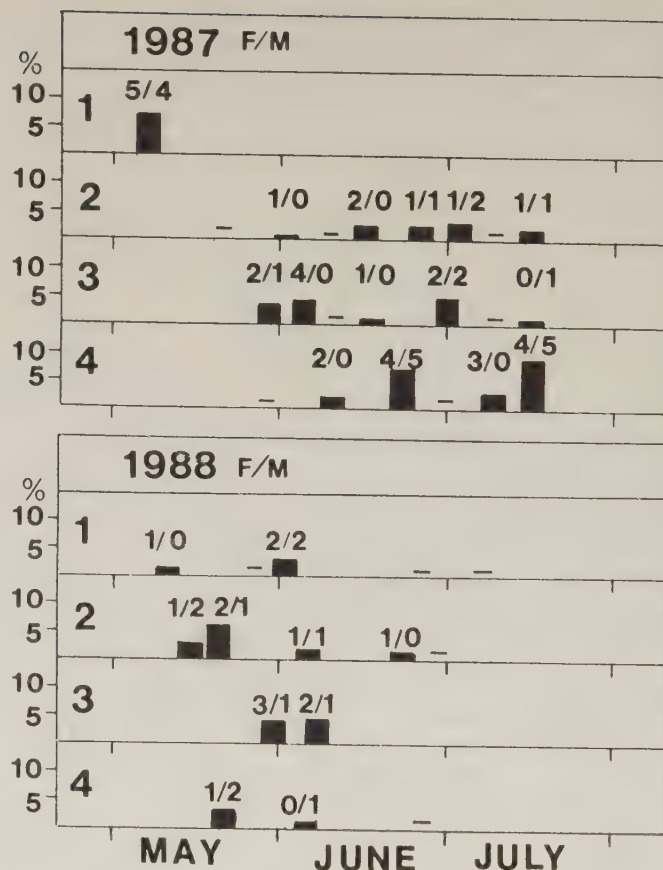


FIG. 9. Percentage occurrence of 2-yr-old recruit spawners (gonad stages 4/5 and 6) in the trap net samples during the reproductive period at locations 1, 2, 3, and 4 in 1987 and 1988. Total numbers of females and males (F/M) are given in each column.

TABLE 3. Length, gonad weight, gonadosomatic index (GSI), and condition factor of 2-yr-old male, female (gonad stages 4/5 and 6), and juvenile herring in the spawning shoals at study locations 1, 2, 3, and 4 (years 1987 and 1988 combined).

	Length (cm)	Gonad wt (g)	GSI	Condition factor
Males				
Mean	14.6	1.56	0.08	0.61
SD	0.6	0.88	0.04	0.04
Range	12.9–15.5	0.42–4.35	0.03–0.18	0.54–0.66
<i>N</i>	19	19	19	19
Females				
Mean	14.4	1.48	0.08	0.60
SD	0.7	0.92	0.04	0.04
Range	13.1–15.8	0.19–3.51	0.02–0.17	0.51–0.70
<i>N</i>	25	16	16	49
Juveniles				
Mean	14.6	0	0	0.54
SD	0.6			0.03
Range	13.8–15.9			0.49–0.58
<i>N</i>	12	12	12	12
Kruskal–Wallis				
<i>H</i>	1.627			13.935
<i>df</i>	2			2
<i>p</i>	>0.10			<0.001

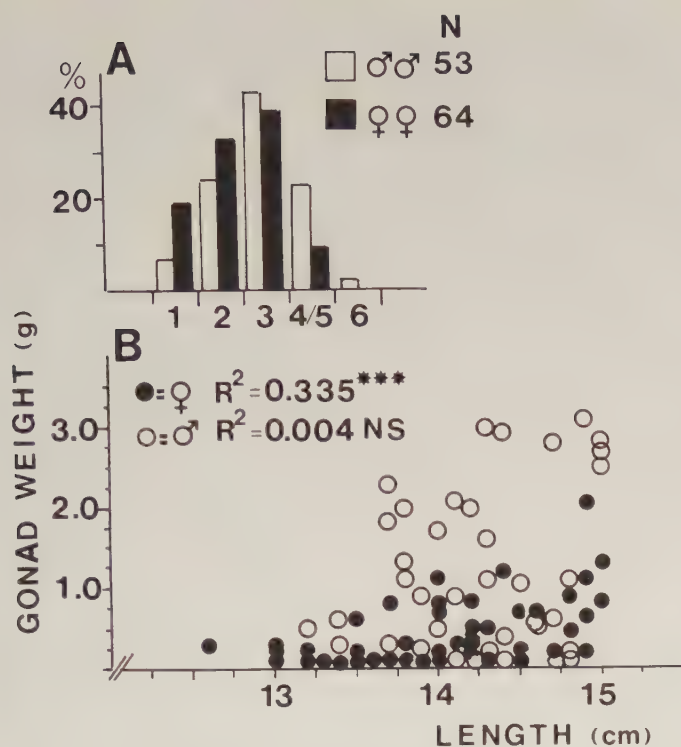


FIG. 10. (A) Frequencies of the gonad developmental stages and (B) gonad weight (data $\log(x)$ transformed prior to analysis) in relation to fish length in 2-yr-old herring in December 1988. ***Significant at $p < 0.001$.

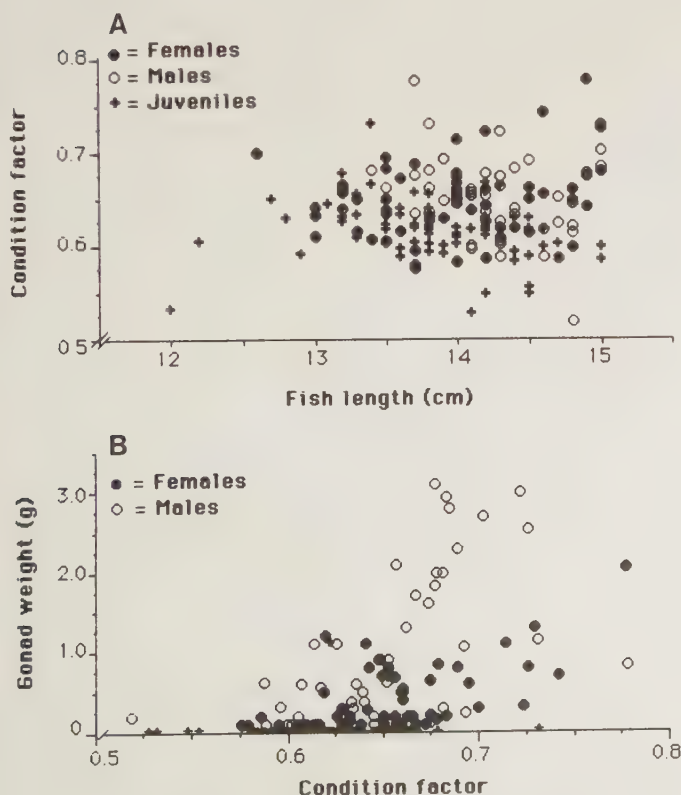


FIG. 11. (A) Condition factor in relation to fish length and (B) gonad weight in relation to condition factor in 2-yr-old herring in December 1988 (locations A–E).

males, but some individuals had gonad weights more than 5% of total fish weight, and in most individuals the development of the ovaries had clearly started. In females, the gonad weight and GSI were positively correlated with fish length (gonad weight: $r = 0.578$; $df = 52$; $p < 0.001$; GSI: $r = 0.468$; $df = 52$; $p < 0.001$), but in males, the correlations were insignificant ($r = 0.063$; $df = 46$; $p > 0.10$ and $r = 0.000$; $df = 46$; $p > 0.10$, respectively) (Fig. 10B).

Fat content of dorsal muscle in fish smaller than 15 cm in December was low, but with a wide range (2.7–5.8%; Fig. 5A). Similarly, fat content in fish collected 2 mo later (February 1988) near the spawning grounds varied between 1.8 and 7.3% (mean = 4.1%; $N = 10$). The amount of fat deposited around the gut was negatively correlated with gonad weight, as in older spawners ($r_s = -0.237$; $p < 0.01$; $N = 184$). Condition factor in winter showed a highly varying pattern, with no correlation with fish length in males ($r = 0.155$; $df = 51$; $p > 0.10$), females ($r = 0.141$; $df = 62$; $p > 0.10$), or juveniles ($r = 0.212$; $df = 72$; $p > 0.10$) (see Fig. 11A). Gonad weight (data $\log(x)$ transformed prior to analyses) increased with the increase of the condition factor (Fig. 11B), which explained 25% of the variation in the gonad weights in males ($df = 46$; $p < 0.001$) and 32.9% in females ($df = 53$; $p < 0.001$).

Discussion

Spawning Time of the Archipelago Sea Herring: Fixed or Flexible?

Individual variation in the factors studied was conspicuous in all samples collected by trawl in December. According to Aneer (1978), schools of Baltic herring disperse during feeding. Because fish feed throughout the winter in the study area, it is possible that they do not form permanent schools but mostly occur dispersed around the area. Consequently, the variation in maturation stages and other parameters studied probably indicates differences between individuals and not between fish schools.

Male herring mature faster than females in the Archipelago Sea, as shown elsewhere (Petursson and Rosenberg 1982; Joensuu and Hahtonen 1984; Ware and Tanasichuk 1989). In December the percentage of males having well-developed gonads was higher than that of females, although in all sampling areas there was a mixture of fish in almost all gonad stages, and in this respect there was no difference between the sexes. A similar pattern was detected around the overwintering area. In adults, the observed gonad stage pattern may be a reflection of the patterns of timing of spawning in a mixed population (i.e. fish which have spawned at different times in the previous spawning period use common feeding and overwintering areas). Thus, individuals with well-developed gonads may represent the early spawners, and less-developed gonads represent late spawners. However, the same pattern was found in 2-yr-old recruit spawners.

There are two alternative concepts for herring maturation: (1) each individual maturing at time (A1) for the first time reproduces the next year again at time (A1) and continues this through its reproductive age, or (2) each individual follows its own maturation cycle, independently of the previous spawning time; first reproduction at time (A1) may well be followed by reproductions at times (A1), (A2), and (A3) in the following years, and so on. The first case represents a highly determined system, where the maturation cycle needs 1 yr to be completed (i.e. genetically fixed system). The analysis of 2-yr-old recruit

spawners in the present study shows that recruitment into the spawning population takes place during the whole spawning period. The key question here, then, is what determines the sequence of the recruitment within the reproductive continuum. Do individuals mature according to their hatching sequence or at random, independently of the hatching time? The first alternative requires a constant maturation rate, so that after hatching it takes a certain time to attain first maturity, the next reproductions following this schedule. There is no doubt that in the development of sexual products, some minimum time is needed for eggs or sperm to mature ready for spawning and this period cannot be shortened because of physiological factors. However, the reproductive traits of fish are generally flexible. First maturation in herring is usually linked with length (Adams 1979), and the later maturation is influenced by food supply and temperature (Aneer 1985; Hay 1985; Hay and Brett 1988; Ware and Tanasichuk 1989). The wave spawning system found in Atlantic (*Clupea harengus harengus*), Pacific, or Baltic herring shows a consistent pattern (Lambert 1987), but the regularity in the observed spawning times does not necessarily mean that individual fish spawn at a fixed time. Hellevaara (1912), who studied Baltic herring scales and otoliths, suggested that some "spring-spawners" had sometimes spawned in the autumn and vice versa. Anokhina (1971) reported a similar shift of the spawning time in Baltic herring. These studies support the idea that the spawning time of an individual is not fixed but can vary within the potential reproductive period.

Ware and Tanasichuk (1989) have shown that the maturation rate of Pacific herring is size dependent, the large fish maturing first. In the Archipelago Sea, all spawning shoals consisted of fish of different sizes which is exceptional compared with many other areas. In Atlantic and Pacific herring, the first spawners of the season are the largest fish, the smaller fish coming only later (Hay 1985; Lambert 1987). In the spawning stock of the Archipelago Sea, mean length of fish varied within the spawning period, but without any consistent trend. In 1987, the first shoals arriving in May consisted of a high number of small fish, which resulted in low mean length in the sample. In 1988, however, the proportion of large fish was highest in shoals arriving first, but this was true only for females, not for males. In all, the shoals were not sorted by size, i.e. although some shoals consisted of more large fish than some others, there were also small fish present. Recruitment into the spawning population did not take place according to fish length. Two-year-old spawning fish varied in length (Table 3), but their condition factor was higher than that of immature fish, demonstrating the importance of nutritional status in reproduction.

Fat Content and Reproduction

In the present study area, Linko et al. (1985) have shown that the composition of lipids in herring follows that in plankton, which demonstrates the close linking between fat cycle of fish and the quality of the food used. There is a connection between food supply and fat content of the herring (Wilkins 1967; Fraser et al. 1987) and also between food supply, fat content, and maturation rate (Hay et al. 1988; Henderson and Almatar 1989).

Fat storages seem to serve the reproductive functions in a different way, depending on the location of the deposits. Fat in the gut mesenteries is more labile than that in the muscles (Blaxter and Holliday 1963), and it probably becomes used in processing the gonad products, as was shown also by the

reversed relation between gut fat and gonad weight in the present study. Fat deposited in the muscle tissue no doubt serves in locomotion, but for instance in capelin (*Mallotus villosus*), as much as one third of the muscle fat is mobilized in developing the gonads (Henderson et al. 1984). The nonlinear trend between muscle fat content and gonad weight found here may demonstrate the character of the maturation process: fat is not metabolized continuously but in a stepwise manner from the fat storages into reproduction.

Reproductive output expressed as gonad weight varied largely between the individuals in the trap net samples, but fish length accounted for this variation only partly. Early spawners had somewhat higher gonad weight than the late ones. In females, the difference can be explained by larger egg size, as the preliminary analysis of fecundity revealed no differences in egg numbers but the early spawners had larger eggs than the late spawners (M. Rajasilta et al., unpubl. data). However, in males, this explanation does not fit. Males spawning early were in a better condition than the late spawning ones, although in females, no differences were found. In winter, fat content and condition factor were positively correlated with gonad weight, which could indicate that the maturation rate is dependent on fish condition: fish which feed well mature at a higher rate and produce larger gonads than those which feed less well, and it is these fish which reproduce first. At time of spawning, the early spawners may still have more energy left than the late spawners because their nutritional status was already better in winter. Hay and Brett (1988) have shown that unfed herring females have the highest GSI's, i.e. in poor feeding conditions, females allocate more energy to the ovaries in relation to the body weight than in good feeding conditions. In the present study, this was not so, however, but the early spawners had higher GSI's and also higher fat content than the late spawning fish.

Fat content, like the other parameters, varied among individuals of both sexes. Fat content correlated positively with fish length, but variation was high between fish of similar size. Large fish did not always have more fat than the smaller ones. Fat content also varied in 2-yr-old fish, although the absolute values were lower than in larger fish. Spawning fish could be identified as early or late spawners according to muscle fat content: fish that spawned early still had plenty of fat left in the muscles; late spawners had low fat reserves. The muscle fat content thus showed a highly variable pattern in winter but a distinct one in the spawning season. Is the fat percentage of the muscle then an indicator of the spawning time or does it only indicate different metabolic demands of the spawning migration?

The differences in the fat content were large, particularly between early and late spawners and between individuals in the winter. Fish which had only 3–4% in their muscles in December probably could not increase the amount to a level of 8–10%, which some individuals had when spawning. Food resources are scarce during the ice-cover period, and food intake of fish is at a low level. It may be that the fat content indicates the time of spawning: those fish having high fat reserves already in December become early spawners and those having low fat content spawn later. These fish could continue food uptake in the spring and early summer, completing the fat reserves until they exceed some critical level and spawn after that (see also Petursson and Rosenberg 1982).

If so, the feeding hypothesis formulated by Aneer (1985) would explain the reproductive pattern of the herring in the study area. High variation between individuals, observable

already before the age of first maturation, supports the idea that each individual follows its own maturation cycle, which is primarily regulated by food, although modified by other ecological factors like seawater temperatures prior to spawning (M. Rajasilta et al., unpubl. data). The synchronization of reproduction, which "collects" the maturing fish together, may then take place by means of chemical communication, e.g. by sexual hormones and pheromones (Liley 1982). The presence of nonreproductive individuals in the spawning shoals may be evidence of migratory instincts triggered in immature fish by mature fish.

The trawl samples were each collected in a rather small area, but in all of them, high between-individual variation was found in gonad stages and fat contents of fish. Fish living in the same area use common food resources and unless these are unlimited in amount, partitioning of food between individuals takes place by competitive means. Although fat accumulation may be a function of fish size and dependent on physiological factors, food intake is also dependent on the foraging success of individual fish. The variable pattern in fat content may be a sign of genetic differences in, for example, foraging abilities, because fish length accounted for the variation of fat only partly. It is possible that within a herring population, some individuals are just better at finding and catching prey, this ability increasing with experience like in some other fish (e.g. Godin 1978).

Unequal partitioning of food resources between individuals may thus bring about differences in nutritional status. The correlations with size or age would then be only secondary, both indicating primarily the increasing ability to find and handle the prey. If the "foraging abilities" vary on a genetical basis, feeding success should increase with age and experience in all individuals, resulting in a positive correlation with those variables which indicate it, but the correlation would not be very strong because of the between-individual differences within the population. If maturation of herring follows this system, the population does not mature at the same time but gradually, which brings about the reproductive continuum. It could be predicted that although all fish of the population would start their maturation cycle contemporarily and from the same energetic level, a diversification would result in the maturation stages later. The selective advantage of this system is that fish by avoiding simultaneous reproduction spread offspring production over a longer time span which diminishes food competition in newly hatched larvae (see also Lambert 1984),

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Cadmium and Zinc Uptake by Two Species of Aquatic Invertebrate Predators from Dietary and Aqueous Sources

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Cadmium and Zn uptake rates via food and water were determined under laboratory conditions for two species of freshwater invertebrate predators. Water mites (*Limnesia maculata*) and caddisfly larvae (*Mystacides* spp.) were exposed for 4 wk to either contaminated chironomid larvae (*Chironomus riparius*, 288–639 $\mu\text{g Cd}\cdot\text{g}^{-1}$ or 778–1152 $\mu\text{g Zn}\cdot\text{g}^{-1}$) or contaminated water (0.1 mg Cd·L⁻¹ or 1.0 mg Zn·L⁻¹). Cadmium was readily accumulated in the two species from both dietary and aqueous sources. A clear difference between exposed and untreated organisms was established. Zinc uptake was generally lower than that of Cd, resulting in small differences between exposed and nonexposed organisms. Cadmium uptake from food and Zn uptake from water dominated in both species. It is concluded that, in addition to uptake of free metal ions from aqueous sources, invertebrate predators can accumulate trace metals from their food. This is an underestimated source of contamination for freshwater invertebrate predators. Changes in internal metal concentrations in the predators are described with a first-order one-compartment uptake model. This model was appropriate where steady-state conditions were approached. When uptake continued throughout the experimental period, uptake rate constants were estimated using linear regression.

Les taux d'absorption du Cd et du Zn à partir de la nourriture et de l'eau ont été déterminées en laboratoire chez deux invertébrés prédateurs d'eau douce. Des hydrachnes (*Limnesia maculata*) et des phryganes (*Mystacides* spp.) ont été exposées pendant 4 sem à des larves de chironomidés contaminées (*Chironomus riparius*, 288–639 $\mu\text{g Cd}\cdot\text{g}^{-1}$ ou 778–1152 $\mu\text{g Zn}\cdot\text{g}^{-1}$) ou à de l'eau contaminée (0,1 mg Cd·L⁻¹ ou 1,0 mg Zn·L⁻¹). Le Cd présent dans les deux sources de contamination s'accumulait directement chez les deux espèces. Une nette différence a pu être établie entre les animaux exposés et témoins. De façon générale, l'absorption du Zn a été moins importante que celle du Cd de sorte que les écarts étaient faibles entre les animaux exposés et les témoins. L'absorption du Cd à partir de la nourriture et du Zn à partir de l'eau ont dominé chez les deux espèces. Les auteurs concluent que les invertébrés prédateurs peuvent absorber les métaux présents à l'état de traces dans leur nourriture, en plus de les absorber à partir de l'eau sous forme d'ions métalliques libres. L'absorption à partir de la nourriture est une source de contamination sous-estimée dans le cas de ces prédateurs. Les variations des concentrations internes de métaux chez les prédateurs sont décrites à l'aide d'un modèle à un compartiment d'ordre premier. Le modèle s'est avéré approprié à l'approche des conditions à l'état d'équilibre. Les constantes du taux d'absorption ont été estimées par régression linéaire lorsque l'absorption a été continue au cours de la période d'essai.

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As a result of the long-term discharge of trace metals from industrial and urban sources, many freshwater ecosystems are highly contaminated. Studies on the transfer of trace metals along food chains comprising algae/bacteria, herbivores, and vertebrate predators have been conducted in both freshwater (Patrick and Loutit 1976; Ferard et al. 1983; Dallinger and Kautzky 1985; Dallinger et al. 1987) and marine environments (Benayoun et al. 1974; Jennings and Rainbow 1979; Davies et al. 1981). Invertebrate predators are seldom included in such studies (Giesy et al. 1980). Furthermore, measurements of trace metal uptake rates in and effects on

invertebrate predator species are scarce (Brown and Pascoe 1988; Clements et al. 1988; McCahon et al. 1989).

No consensus exists in the literature on the relative importance of trace metal uptake from dietary or aqueous sources in aquatic organisms (e.g. Cd, Kay 1985). In some studies, it is reported that the main route of trace metal accumulation is through uptake of free metal ions or organometal complexes from water (Williams and Giesy 1978; Taylor 1983; van Hattum et al. 1989). Other authors suggest food as the predominant source (Davies et al. 1981; Leland and Kuwabara 1985; Dallinger et al. 1987). Most information on the subject is available for marine invertebrates (Borchardt 1983; Riisgård et al. 1987). Only limited information is available for their freshwater counterparts (Mummert 1987; Hatakeyama and Yasuno 1987).

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In this study, uptake rates and routes of Cd, a nonessential metal, and Zn, an essential metal, were compared and contrasted in water mites (*Limnesia maculata*) and caddisfly larvae (*Mystacides* spp.) using chironomid larvae (*Chironomus riparius* (Meigen)) as food. In a comparison of trace metal uptake in both species, effects of differences in food handling were included.

Materials and Methods

Experimental Organisms

Water mites and caddisfly larvae were collected on sandy substrates in the littoral zone of Lake Maarsseveen I (52°09'N, 5°04'E), The Netherlands. Water mites were identified as *Limnesia maculata* (Müller 1776). Two species of (case-bearing) caddisfly larvae, *Mystacides longicornis* (L.) and *M. nigra* (L.), were collected. No discrimination was made between these two species when alive, but all individuals were identified at the end of the experiments. Results showed no significant differences in uptake of either metal between the two species (*t*-test, $p > 0.1$). This, together with observations that the species had similar ecological preferences in the field, gave reason to combine the two species of caddisfly larvae in further analyses. Both species of caddisfly larvae (J. Kuipers, Department of Aquatic Ecology, University of Amsterdam, pers. comm.) and watermites (Ten Winkel et al. 1989) prey upon chironomid larvae. Subsequently, organisms used in this study will be referred to as "water mites" or "caddisfly larvae".

Water mites and caddisfly larvae were acclimated for at least 2 wk to the experimental conditions used, i.e. a temperature of $15 \pm 1^\circ\text{C}$, a 16:8 h day–night light regime, and food consisting of chironomid larvae (*C. riparius*) from a laboratory culture. In all experiments, aerated Lake Maarsseveen I water was used (for typical water parameters, see Timmermans et al. 1989), both for blanks and preparation of metal-contaminated water.

Exposure via Food

Five water mites consumed approximately one chironomid larva per day. Water mites could handle third or early fourth instar chironomid larvae (average 0.50–0.65 mg dry weight). Upon encounter, a water mite would pierce a chironomid larva, inject digestive fluids, and suck a few segments of the chironomid body dry (external digestion). Total consumption of a chironomid larva normally took several hours, after which time only the exoskeleton would remain. Uneaten remains of chironomid larvae were collected daily upon water renewal and/or addition of new food. An individual caddisfly larva captured and consumed approximately one full-grown fourth instar chironomid larva (average 0.8–1.0 mg dry weight) per day. Consumption of a chironomid usually took less than 1 h.

The experiments were performed in small glass flasks (25 mL). Each flask contained three to five water mites or one caddisfly larva, providing sufficient biological material for Cd or Zn analyses. Water in the flasks was changed three times a week. Water samples were taken for trace metal analyses before and after renewal. These samples were acidified to pH 1.0 with 1 N HNO_3 (Baker Ultrex) and stored until analysis.

During the 4-wk experimental period, water mites and caddisfly larvae were fed with Cd- or Zn-contaminated larvae or untreated chironomid larvae. After exposure varying from 0 to 32 d (water mites) or 0 to 28 d (caddisfly larvae), the organisms were killed and replicate samples stored at -20°C prior to analysis.

Preparation of Contaminated Food

Second instar chironomid larvae from the laboratory culture were exposed in a semistatic experiment to $0.1 \text{ mg Cd}\cdot\text{L}^{-1}$ or $1.0 \text{ mg Zn}\cdot\text{L}^{-1}$ for 2 wk prior to feeding them to predators. These concentrations were identical to exposure of predators via water (see Exposure via Water for details). The treatments were performed in polyethylene containers ($20 \times 10 \times 6 \text{ cm}$). The test solution (1 L) was renewed three times per week. Samples were taken before and directly after renewal to verify the Cd or Zn concentrations. These samples were acidified to pH 1.0 with 1 N HNO_3 (Baker Ultrex) and stored until analysis. By the end of exposure, larvae were mostly in the third instar stage, and to prevent further development, they were then transferred to water at 10°C .

On all occasions of feeding on chironomids by predators, samples of untreated and contaminated chironomid larvae were taken. Contaminated chironomid larvae fed to water mites had higher Cd and Zn concentrations than those fed to caddisfly larvae (Table 1). The explanation for this is uncertain: perhaps starting with somewhat younger chironomid larvae in the preparation as contaminated food for water mites caused this difference. Cadmium concentrations in exposed chironomid larvae were 500–1000 times higher than those in control individuals whereas Zn concentrations in contaminated larvae were only 2–4 times those in controls. Cadmium and Zn concentrations in chironomids fed to both water mites and caddisfly larvae varied somewhat over time, as indicated by the size of the standard error about the means in Table 1. Elevated metal concentrations in chironomids did not result in a higher mortality (data not shown). No leaching from contaminated chironomids to water was measured; Cd and Zn concentrations in water remained below limits of detection (Table 1). No significant decrease in metal concentrations in intact chironomids was observed during the time they were being presented to predators (data not shown).

Exposure via Water

Exposure via water was performed during the same period as the feeding experiments. Water mites and caddisfly larvae were separately exposed in a semistatic experimental set-up to either $0.1 \text{ mg Cd}\cdot\text{L}^{-1}$, $1.0 \text{ mg Zn}\cdot\text{L}^{-1}$, or clean water in polycarbonate aquaria ($35 \times 20 \times 20 \text{ cm}$). Test solutions were prepared from Lake Maarsseveen I water by dilution of a stock solution of $1000 \text{ mg Cd}\cdot\text{L}^{-1}$ (as CdCl_2 , Merck-Tritisol) or $1000 \text{ mg Zn}\cdot\text{L}^{-1}$ (as ZnCl_2 , Merck-Tritisol).

After exposure varying from 0 to 32 d (water mites) or 0 to 28 d (caddisfly larvae), animals were removed from their aquaria and replicate samples stored at -20°C prior to analysis.

Predators were fed clean chironomid larvae from the laboratory culture. Since food organisms could absorb Cd or Zn from the exposure water before they were eaten, a pilot experiment was performed. It demonstrated that no measurable increase in Cd or Zn concentrations in the food organisms could be detected within 2 h (data not shown). Caddisfly larvae usually consumed their prey within an hour, and uneaten remains of chironomids were removed after 2 h. In the case of water mites, total consumption of chironomid larvae required several hours, but as only the interior of chironomids was consumed, exposure via food was highly unlikely.

The 5-L test solution was renewed three times per week during the experiments. Water samples were taken before and directly after renewal to verify metal concentrations. These samples were acidified to pH 1.0 with 1 N HNO_3 (Baker Ultrex)

TABLE 1. Cadmium and Zn concentrations in exposure media. (A) Concentrations in chironomid larvae used in exposure via food experiments (average $\pm$ SE in $\mu\text{g}\cdot\text{g}$ dry weight $^{-1}$, n = approximately 15). (B) Concentrations in water during exposure via water (average $\pm$ SE in $\mu\text{g}\cdot\text{L}^{-1}$, n = approximately 15).

Experiment and predator	Cd		Zn	
	Control	Contaminated	Control	Contaminated
(A) Exposure via food				
Water mites	0.51 $\pm$ 0.09	639 $\pm$ 37	320 $\pm$ 9	1152 $\pm$ 55
Caddisfly larvae	0.51 $\pm$ 0.09	288 $\pm$ 24	320 $\pm$ 9	778 $\pm$ 32
(B) Exposure via water				
Water mites	< 0.05	94 $\pm$ 7	< 2.0	985 $\pm$ 15
Caddisfly larvae	< 0.05	85 $\pm$ 11	< 2.0	915 $\pm$ 55

and stored until analysis. Ranges of actual metal concentrations in water were within reasonable limits of nominal concentrations (Table 1). Concentrations were sublethal, as no significant differences in mortality between exposed and control organisms were observed (t -test, $p > 0.1$).

Analytical Procedures

Caddisfly larvae were removed from their cases with sharp glass rods before analysis. In caddisfly larvae, the gut was examined for undigested chironomids. This inspection was impossible for water mites, as they have no guts.

Individual caddisfly larvae, pooled water mites (three to five individuals) and pooled chironomid larvae (approximately three individuals) were lyophilized, weighed, and digested in 0.2 mL of concentrated HNO_3 (70%, Baker Ultrex) in 2.2-mL polyethylene tubes. Nitric acid was evaporated at 80°C overnight, after which time 0.2 mL of H_2O_2 (30%, Merck) was added to the residue. After evaporation of H_2O_2 , the residue was redissolved in 1.0–2.0 mL 0.1 N HNO_3 (Baker Ultrex), depending on expected metal concentrations and the chosen detection method (flame or graphite furnace atomic absorption spectrometry). Acidified water samples were analysed without further pretreatment.

Cadmium in control organisms and blank water samples was analysed with graphite furnace atomic absorption spectrometry equipped with a deuterium background correction (Perkin-Elmer 1100B, with HGA 400 and AS-40). Standard addition calibration was performed for biological materials, as interfering matrix effects were observed. Exposed organisms (in the case of Cd), all exposed and control organisms in Zn experiments, and Cd- or Zn-contaminated water samples were analysed with flame atomic absorption spectrometry (Perkin-Elmer 1100B), equipped with an impact bead.

Quality control of trace metal analyses was performed by analysing digested blanks and reference materials (IAEA shrimp MA-A-3/TM and standard water IAEA W4). Cadmium results in shrimp samples ranged from 92 to 107% of the certified value, while for Zn, values ranged from 89 to 109%.

Models Used

To fit curves to the uptake data, one of two methods was used, depending on whether concentrations in predators appeared to reach an asymptote or continued increasing indefinitely. In the first case, a first-order one-compartment uptake model was utilized. Basic assumptions of the model are that uptake and elimination rates remain constant and that constant

concentrations in food and water are supplied during exposure. In its integrated form, the uptake model can be written as

$$(1) \quad C_{\text{pred}} = \frac{k_1 C_w + aRC_f}{k_2} (1 - e^{-k_2 t})$$

which is equal to

$$(2) \quad C_{\text{pred}} = C_{\text{ss}} (1 - e^{-k_2 t})$$

where C_{pred} = Cd or Zn concentration (micrograms per gram dry weight) in water mites or caddisfly larvae, C_{ss} = Cd or Zn concentration (micrograms per gram dry weight) in water mites or caddisfly larvae during steady-state conditions, k_1 = rate constant (per day) for uptake from water, k_2 = rate constant per day for elimination, a = assimilation efficiency from food (micrograms metal absorbed per microgram metal ingested), R = feeding rate (micrograms ingested food per microgram body weight per day), C_w = concentration (micrograms per gram) in water, and C_f = concentration (micrograms per gram) in chironomids.

Our experimental design represented two extremes: either uptake from water ($k_1 C_w$) or uptake via food (aRC_f). Using a two-parameter nonlinear iterative least squares regression procedure, experimental data were fitted to a curve described by equation (2) with SYSTAT. In addition to this, calculations of the following parameters were made: $\text{BCF}_{\text{theor.}}$ = theoretical bioconcentration factor (k_1/k_2), $\text{BMF}_{\text{theor.}}$ = theoretical biomagnification factor ($(aR)/k_2$), and $t_{0.5}$ = biological half-life time (days) ($\ln 2/k_2$).

When no steady-state conditions were approached, linear regression was used to estimate only uptake rate constants. An apparent linear uptake was not considered as being in conflict with presence of an elimination mechanism. The criterion for use of linear regression was a negative estimate of k_2 in the one-compartment model.

An estimate of relative contribution of Cd or Zn uptake via either water or food for predators was made based on the ratio $k_1 C_w : aRC_f$. Furthermore, observed bioconcentration and biomagnification factors were calculated for all experiments, with the observed trace metal values on the last sampling day: $\text{BCF}_{\text{obs.}}$ = observed bioconcentration factor: concentration in predator/concentration in water and $\text{BMF}_{\text{obs.}}$ = observed biomagnification factor: concentration in predator/concentration in prey.

Routine statistical analyses (t -test, Kruskal–Wallis test) were applied according to Sokal and Rohlf (1981) using STATWORKS. Unless stated otherwise, significance was tested at the $p < 0.05$ level.

Results

Exposure via Food

Cadmium concentrations in control water mites remained fairly constant throughout the experimental period, while Zn concentrations increased (Fig. 1A and 1B). Feeding on contaminated chironomid larvae resulted in a significant increase in Cd and Zn concentrations in water mites (Kruskal Wallis, $p < 0.03$). Cadmium concentrations increased for at least the first 20 d (Fig. 1A), while steady-state concentrations were approached in the Zn experiment (Fig. 1B).

A significant difference was observed between caddisfly larvae fed with untreated and Cd-contaminated chironomids (Fig. 1C, Kruskal-Wallis, $p < 0.01$). After an initial increase, Cd concentrations in exposed individuals were almost constant between days 4 and 18, after which time concentrations rose again, without signs of reaching a steady state. For Zn, less distinct differences between exposed and nonexposed organisms were observed. Only during the last two sampling days did significant differences exist between Zn-exposed and control organisms (Fig. 1D, Kruskal-Wallis, $p < 0.05$).

Steady-state conditions in exposed organisms were not reached during the experimental period.

Exposure via Water

Concentrations of Cd in control water mites were constant throughout the experimental period, while Zn concentrations increased slightly in controls (Fig. 2A and 2B). Exposure to aqueous Cd caused a significant increase in Cd concentrations in water mites (Kruskal-Wallis, $p < 0.02$). After an initial rapid increase, Cd concentrations tended to stabilize (Fig. 2A). Upon exposure to Zn-contaminated water, concentrations in water mites increased slightly until day 21, after which time a more substantial increase was observed (Fig. 2B).

Exposure to Cd or Zn resulted in a significant increase in concentrations in caddisfly larvae (Fig. 2C and 2D). In spite of considerable variation in Cd and Zn concentrations in individuals on every sampling day, a significant difference between exposed and nonexposed organisms was observed (Kruskal-Wallis, $p < 0.02$). The initial accumulation of Cd was slow and continued to the end of the experiment. Steady-state conditions were only approached after exposure to Zn.

EXPOSURE VIA FOOD

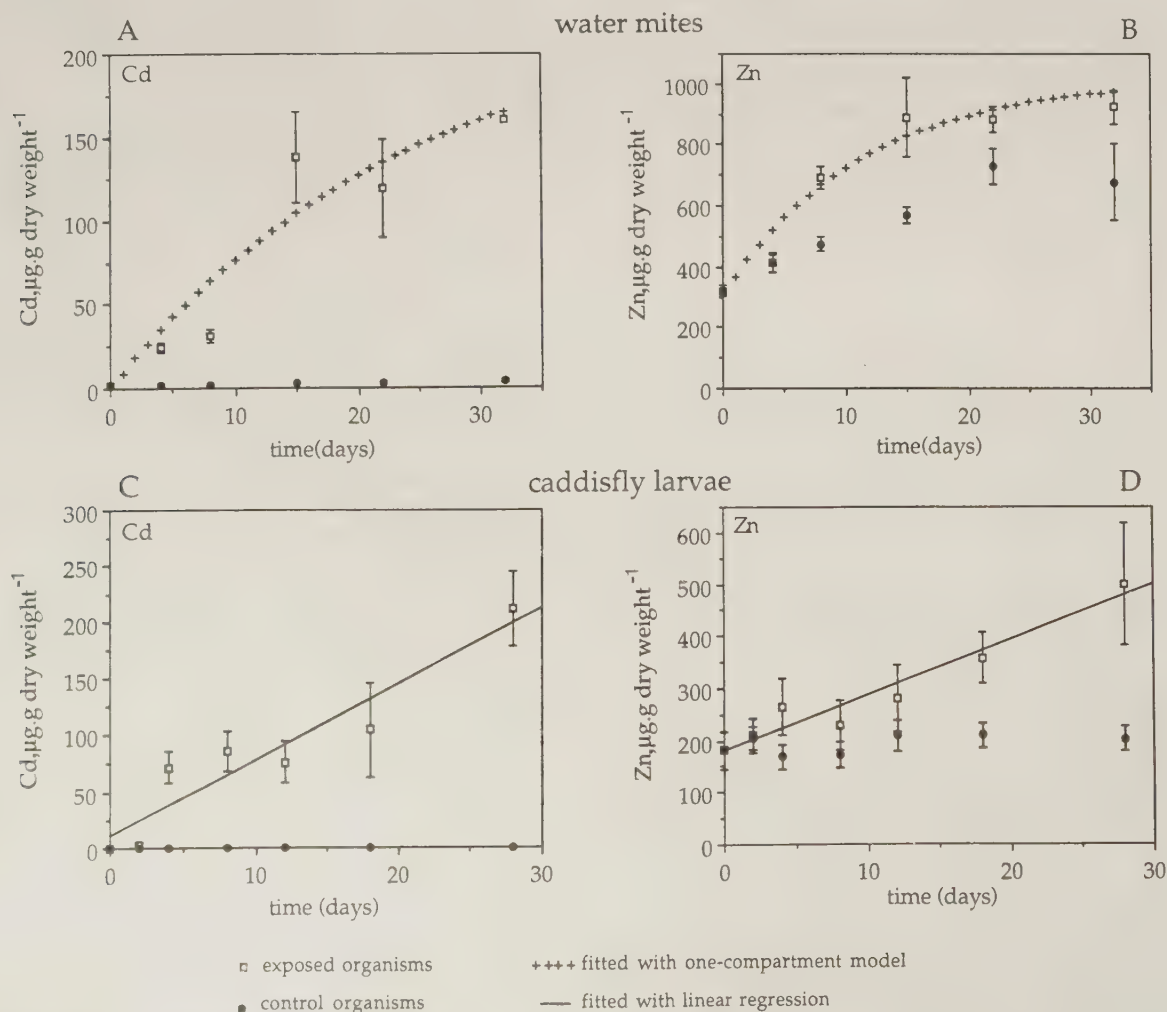


FIG. 1. Cadmium and Zn concentrations (average $\pm$ SE) in (A and B) water mites and (C and D) caddisfly larvae fed with untreated or contaminated chironomid larvae. For exposed water mites, lines with crosses are concentrations predicted from a first-order one-compartment uptake model (Table 2). For exposed caddisfly larvae, solid lines are linear regressions.

EXPOSURE VIA WATER

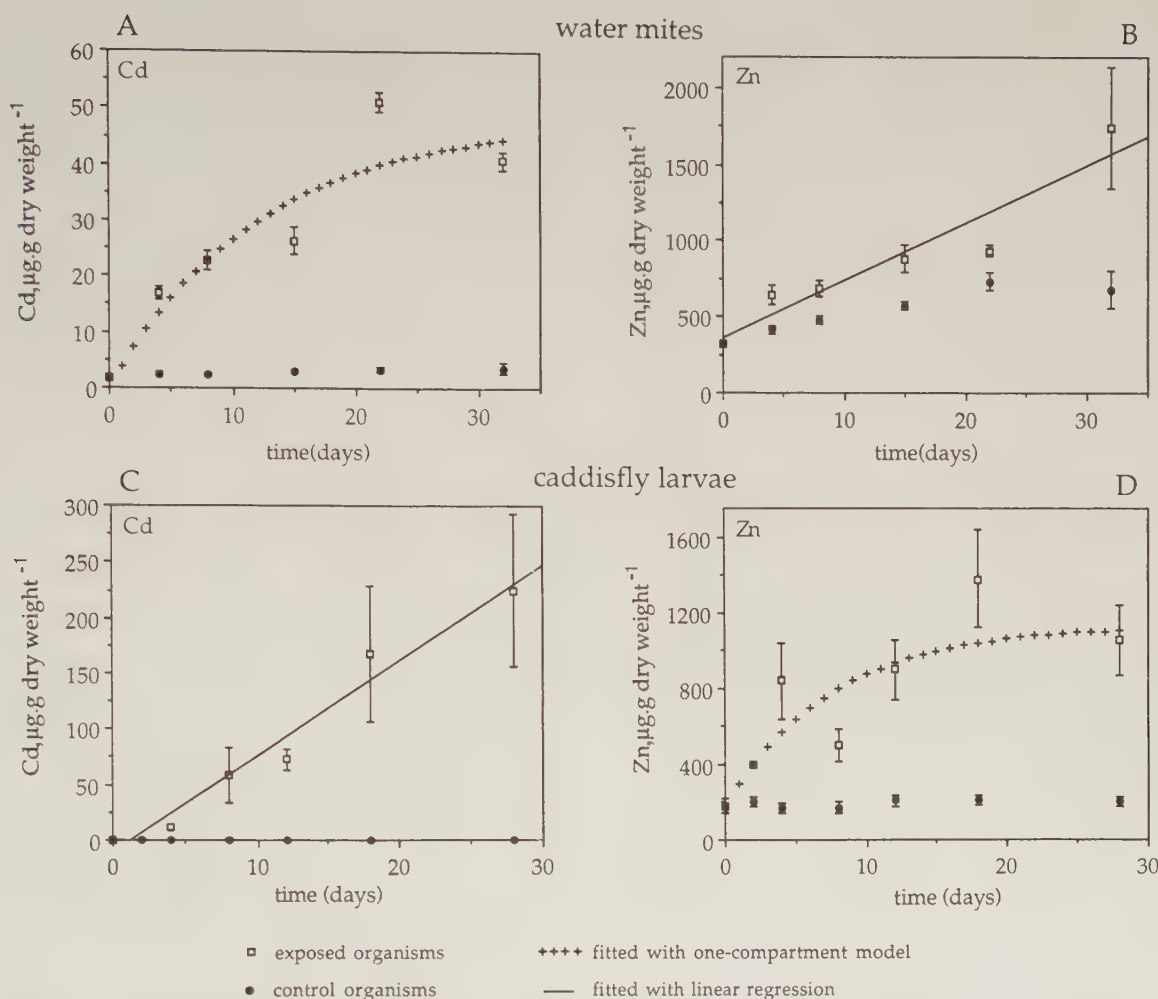


FIG. 2. Cadmium and Zn concentrations (average $\pm$ SE) in (A and B) water mites and (C and D) caddisfly larvae exposed to uncontaminated or contaminated water. For Cd-exposed water mites and Zn-exposed caddisfly larvae, lines with crosses are concentrations predicted from a first-order one-compartment uptake model (Table 2). For Zn-exposed water mites and Cd-exposed caddisfly larvae, solid lines are linear regressions.

Kinetics

With at least a partial fulfillment of assumptions in the model (see Discussion), estimates of uptake and elimination rate constants and derived parameters in exposed water mites and caddisfly larvae were made. In those cases where uptake continued throughout the experimental period, only uptake rate constants and derived parameters were estimated (Table 2).

When trace metal concentrations in water mites approached steady-state conditions (Fig. 1 and 2), the model presented reasonable descriptions of experimental data. Rate constants, k_1 , for uptake of Cd and Zn from water were similar in water mites, but uptake of Zn through water in caddisfly larvae was much higher (Table 2). The route of exposure seemed to influence the biological half-life time, $t_{0.5}$, of Cd and Zn in water mites (Table 2). Based on concentrations in water and contaminated prey (Table 1), uptake rate constants (Table 2), and feeding rate ($R = 0.09 \mu\text{g food} \cdot \mu\text{g body weight}^{-1} \cdot \text{d}^{-1}$) the proportions of uptake by water mites via food and water were estimated. For Zn, uptake via water dominated (65%) while for Cd uptake via food dominated (94%).

The uptake rates, k_1 , of Zn by caddisfly larvae were higher than those for Cd (Table 2). Elimination was only observed in

the case of Zn uptake through water (Fig. 2D), where steady-state conditions appeared to be approached. In all other experiments with caddisfly larvae, uptake continued throughout the experimental period. Calculation of the relative contribution of uptake routes (Tables 1 and 2), using a feeding rate of $0.65 \mu\text{g food} \cdot \mu\text{g body weight}^{-1} \cdot \text{d}^{-1}$, demonstrated a dominance via food in the case of Cd (62%) whereas Zn uptake via water dominated (76%).

Water mites had higher Cd and Zn assimilation efficiencies than did caddisfly larvae. Cadmium uptake in water mites was especially efficient (Table 2).

Discussion

Exposure via Food

Most caddisfly larvae had empty guts, and therefore, interference by undigested food in metal analyses is highly unlikely. Absence of biomagnification, defined as uptake of trace metals via food with the result that tissue concentrations in predators are higher than those in prey, may be caused by the fact that the high Cd concentrations in chironomids could

TABLE 2. Parameter estimates for uptake of Cd or Zn in water mites and caddisfly larvae via food or water. All values (except C_{ss} and $t_{0.5}$) are expressed as average $\pm$ SE. See Materials and Methods (Models Used) for definitions of parameters. n.e. = parameter not estimated or calculation not made as result of the absence of steady-state conditions.

Parameter (unit)	Cd		Zn	
	Water mites	Caddisfly larvae	Water mites	Caddisfly larvae
<i>Exposure via food</i>				
a ($\mu\text{g absorbed} \cdot \mu\text{g ingested}^{-1}$)	0.61 ± 0.11	0.044^a	0.16 ± 0.04	0.069^a
k_2 (d^{-1})	0.042 ± 0.027	n.e.	0.089 ± 0.028	n.e.
$\text{BMF}_{\text{theor.}}$	0.36 ± 0.09	n.e.	0.62 ± 0.37	n.e.
$\text{BMF}_{\text{obs.}}$	0.25 ± 0.07	0.73 ± 0.11	0.80 ± 0.12	0.63 ± 0.07
C_{ss} ($\mu\text{g} \cdot \text{g dry weight}^{-1}$)	224	n.e.	705	n.e.
$t_{0.5}$ (d)	17	n.e.	8	n.e.
<i>Exposure via water</i>				
k_1 (d^{-1})	37 ± 4	56^a	34^a	121 ± 27
k_2 (d^{-1})	0.082 ± 0.015	n.e.	n.e.	0.131 ± 0.042
$\text{BCF}_{\text{theor.}}$	450 ± 32	n.e.	n.e.	924 ± 156
$\text{BCF}_{\text{obs.}}$	455 ± 53	2500 ± 755	1680 ± 53	1050 ± 71
C_{ss} ($\mu\text{g} \cdot \text{g dry weight}^{-1}$)	48	n.e.	n.e.	950
$t_{0.5}$ (d)	9	n.e.	n.e.	5

^aEstimated with linear regression.

not be exceeded. In the case of Zn, regulation may have taken place. Transfer of trace metals to higher trophic levels is, however, realistic, as in both invertebrate predators studied, metal concentrations increased as a result of exposure via contaminated food. Field observations in the Maarsveen Lakes, where higher Cd and Zn concentrations were observed in water mites than in their prey (chironomid larvae) (Timmermans et al. 1989), are consistent with results of the present experiment.

It was assumed that water mites, using external digestion with the resulting incomplete consumption of their prey, would be exposed to lower trace metal concentrations. This was, however, not the case. Studies have revealed that exoskeletons of (contaminated) chironomid larvae contain small amounts of trace metals relative to internal parts (Hare et al. 1991b; Timmermans et al. unpubl. data). The injection of digesting enzymes and subsequent uptake of the digested interior of chironomids obviously was very efficient, especially in the case of Cd (Table 2). Caddisfly larvae, on the contrary, start to eat chironomid larvae from one end, thereby possibly spilling some of the larvae's innards. In this way, caddisfly larvae can be exposed to smaller amounts of trace metals, and this seemed to be reflected in lower uptake efficiencies (Table 2). These results clearly demonstrate that differences in food handling can influence trace metal uptake, both in terms of amounts and efficiencies, in freshwater invertebrate predators. Food handling should therefore be included in interpretation of toxicokinetic data.

Exposure via Water

Bioconcentration, defined as uptake of aqueous metal ions resulting in higher concentrations in water mites and caddisfly larvae than in ambient water, was demonstrated in the experiments. It cannot be excluded that some of the metals reported as accumulated in water mites or caddisfly larvae were in fact externally adsorbed, but this would have been small amounts

(<10%, Timmermans et al., unpubl. data). The presence of bioconcentration parallels findings by Taylor (1983) and Kay (1985) who stated that most organisms accumulate trace metals readily from aqueous sources. Estimated and/or observed BCF values for Cd in water mites and caddisfly larvae lie well above median concentration factors of 100 (on a wet weight basis) as indicated by Taylor (1983).

Relative Contribution of Uptake Routes

For both invertebrate predators used in this study, Cd uptake through food dominated whereas for Zn, uptake through water dominated. These results are similar to those reported by Giesy et al. (1980) and confirm results reported for freshwater vertebrate predators, invertebrate herbivores, and omnivores (Pentreath 1976; Hatakeyama and Yasuno 1987; van Hattum et al. 1989). In many aquatic ecosystems, trace metal concentrations in food organisms are higher than in water (Dallinger et al. 1987). This emphasizes that when it comes to protection of higher trophic levels, trace metal concentrations in food must be considered.

Differences between Cd and Zn uptake during exposure via food or water are typical examples of differences between non-essential (Cd) and essential (Zn) metals (Bryan 1964; White and Rainbow 1982; Rainbow 1988). In both control predators and in control chironomids, Cd concentrations were very low. A large increase in concentrations was observed after either dietary or aqueous exposure. Increases in Zn concentrations were limited and seem to be regulated.

The increase in Zn concentrations in control water mites in both experiments cannot be explained by uptake from water, as trace metal concentrations were extremely low. Exposure via food is therefore most likely: cannibalism was observed and *C. riparius* larvae contained more Zn than in field-collected chironomids (Timmermans et al. 1989).

Kinetics

Uptake models are frequently used for description of uptake kinetics of hydrophobic compounds (Moriarty 1983; Walker

1987). The model describes uptake through water and food at a rate that is maximal at the time of first exposure. In time, elimination will increase, and a steady-state residue concentration will be established. For trace metals, these models are not commonly applied (van Hattum et al. 1989; Hare et al. 1991a).

Basic requirements for use of the first-order one-compartment uptake model, which were that metal concentrations in food and water were constant during the experiments, were partly fulfilled. Cadmium and Zn concentrations in exposed chironomids as well as concentrations in water varied somewhat (Table 1). Conclusive evidence for metal elimination in water mites and caddisfly larvae could not be provided, as no separate elimination experiments were performed. However, since steady-state conditions were approached in a number of cases, elimination probably occurred. In the use of the model, it was assumed that during exposure via food the contribution from water and during exposure via water the contribution from food could be neglected. Cadmium or Zn uptake in uncontaminated chironomid larvae, used as food during exposure via water, is thought to have been minimal based on results of a pilot experiment. Cadmium and Zn concentrations in water during exposure to contaminated food were extremely low (Table 1).

Only when steady-state conditions were approached could the model give a fair description of the experimental data. Apparent linear uptake in some of the experiments is, however, certainly not considered as contradictory to the first-order one-compartment model. If the experiments had lasted longer, especially the experiments with caddisfly larvae, true first-order uptake patterns might have resulted. The delay in accumulation after first exposure (Fig. 1A and 1C) is a more serious deviation. Although sigmoidal uptake patterns have been reported (Brisbin et al. 1990), further experimental work would be necessary to rule out the existence of an artifact.

Biological half-life times indicated that water mites eliminated both Cd and Zn rapidly. The fact that steady-state conditions were only approached in the Zn experiment via water with caddisfly larvae demonstrated that in most cases, metals were strongly retained in this organism.

In spite of the model's general biological realism, it must be realized that some assumptions within the model, such as constant metal concentrations in food and water, are seldom encountered under natural conditions. In the present study, feeding rates could be quantified with precision, but supply of food with constant trace metal concentrations was experimentally impossible. Inevitably, uptake and elimination rate constants will show variation. The estimates therefore will be highly dependent on conditions, making comparisons with other studies almost impossible.

A general point of concern is the absence of physiological data for both water mites and caddisfly larvae. It is known that water mites have the possibility of elimination through excretory glands (cf. kidneys) around the digestive tract (C. Davids, Department of Aquatic Ecology, University of Amsterdam, pers. comm.), but the exact nature of this phenomenon is unknown. Caddisfly larvae too have possibilities of excretion by means of their Malpighian tubules (Hickin 1967). Further physiological research is therefore indispensable for conclusive statements on trace metal elimination in these species. Given the absence of these data, the information presented here must be regarded as a first attempt to quantify uptake and elimination rate constants for aquatic invertebrate predators.

In summary, it may be concluded from this study that (1) in addition to uptake of metals from water, water mites and cad-

disfly larvae can accumulate Cd and Zn from their food, (2) a first-order one-compartment uptake model gave a reasonable description of experimental data when steady-state conditions were approached, (3) clear differences between exposed and control organisms were observed in the case of Cd (for Zn, less distinct differences were observed), and (4) differences in food handling should be considered in any interpretation of toxicokinetic data of predatory organisms.

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Zooplankton Retained in Sequential Sediment Traps along the Beaufort Sea Shelf Break during Winter

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Zooplankton retained in four sediment traps deployed along the shelf break of the eastern Beaufort Sea, from September 1987 to March 1988, were used to investigate temporal and regional variations of the zooplankton community during winter. Despite trap selectivity, the species composition indicated that both the shelf community and Atlantic water community of the deep Arctic Ocean are excluded from the shelf break at this time of year. There was no evidence of off-shelf transport during the study period. Taxa collected, predominantly pteropods (*Spiratella helicina*) and calanoid copepods, were typical of the community in the upper 200 m of the central Arctic Ocean. The abundance of pteropods was strongly associated with ice cover. The easternmost trap, located at the entrance to Amundsen Gulf, had a distribution of animals distinct from those in the other traps. It lay outside the influence of the Beaufort Gyre and Beaufort Undercurrent, which apparently affected the other locations.

Le zooplancton retenu dans quatre pièges à sédiments installés le long de la rupture de pente de l'est de la mer de Beaufort, de septembre 1987 à mars 1988, a été étudié pour analyser les variations temporelles et régionales de la communauté de zooplancton pendant l'hiver. Malgré la sélectivité des pièges, la composition des espèces indiquait que la communauté du plateau continental et celle des eaux de l'Atlantique de la partie profonde de l'océan Arctique ne sont pas présentes dans la zone de la rupture de pente à ce temps de l'année. Rien n'indique qu'il y ait déplacement de la zone au-delà du plateau continental. Les taxons recueillis, surtout des ptéropodes (*Spiratella helicina*) et des copépodes calanoïdes, étaient typiques de la communauté des 200 m supérieurs de la partie centrale de l'océan Arctique. L'abondance de ptéropodes présentait une forte association avec la couverture de glace. Le piège le plus à l'est, placé à l'entrée du golfe Amundsen, présentait une distribution d'espèces animales distincte de celle des autres pièges. Il était placé au-delà de la zone d'influence de la circulation de Beaufort et du courant sous-marin de Beaufort, qui avait apparemment des effets sur les pièges des autres emplacements.

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The distribution of zooplankton in the eastern Beaufort Sea at times of year other than summer is not well established. Woods and Smiley (1987) list only eight sets of data collected between 1914 and 1985 that include information on zooplankton during the months from October to June. Winter data from the continental shelf of the Beaufort Sea were collected in the vicinity of Issungnak artificial island (Erickson et al. 1983; Pett et al. 1983) and from shallow nearshore waters (F. F. Slaney and Co. Ltd. 1974; Evans and Grainger 1980). Farther north in the Arctic Ocean, community composition and depth distribution of zooplankton have been observed in winter from drifting ice islands (Johnson 1963; Hopkins 1969; Kobayashi 1974; Rudyakov 1983; Kosobokova 1986). Recently, Hargrave et al. (1989) provided winter and spring data on the zooplankton community over the continental shelf off Axel Heiberg Island using a sequential trap suspended 100 m below seasonal pack ice.

Here we present zooplankton data collected by four sequential traps moored throughout winter along the shelf break of the Canadian Beaufort Sea. It was not our specific intention to sample zooplankton, nor are sequential traps specifically adapted

to such a task. However, we have been provided fortuitously with a rare set of four simultaneous, successive records of zooplankton from this particularly difficult to sample environment.

Methods

As part of an interdisciplinary study of shelf-exchange processes in the eastern Beaufort Sea, we moored four sequential sediment traps from August 1987 to March 1988. The four moorings were spaced approximately equidistantly from the entrance to Amundsen Gulf, just east of Cape Bathurst, west to Mackenzie Canyon (Fig. 1), in water depths from 170 to 200 m. Each mooring consisted of a sequential sediment trap, 55 m above the bottom, and two recording current meters (Aanderaa, model RCM-4) (Table 1). The upper current meter on each mooring was at 35 m depth; the lower was just below the trap. Traps SS1 and SS3 were Parflux Mark 6 traps (Honjo and Doherty 1988), with a trap opening of 0.509 m² and 13 receiving cups. Traps SS2 and SS4 were S³T traps (Baker and Milburn 1983), with a collection area of 0.032 m² and 10 cups. The traps were deployed at the end of August 1987 and began collecting on 1 September (31 August at SS2), with a collection interval of 16 d for each receiving cup. The final collection was completed on 7 February 1988 at SS2, 8 February 1988 at SS3,

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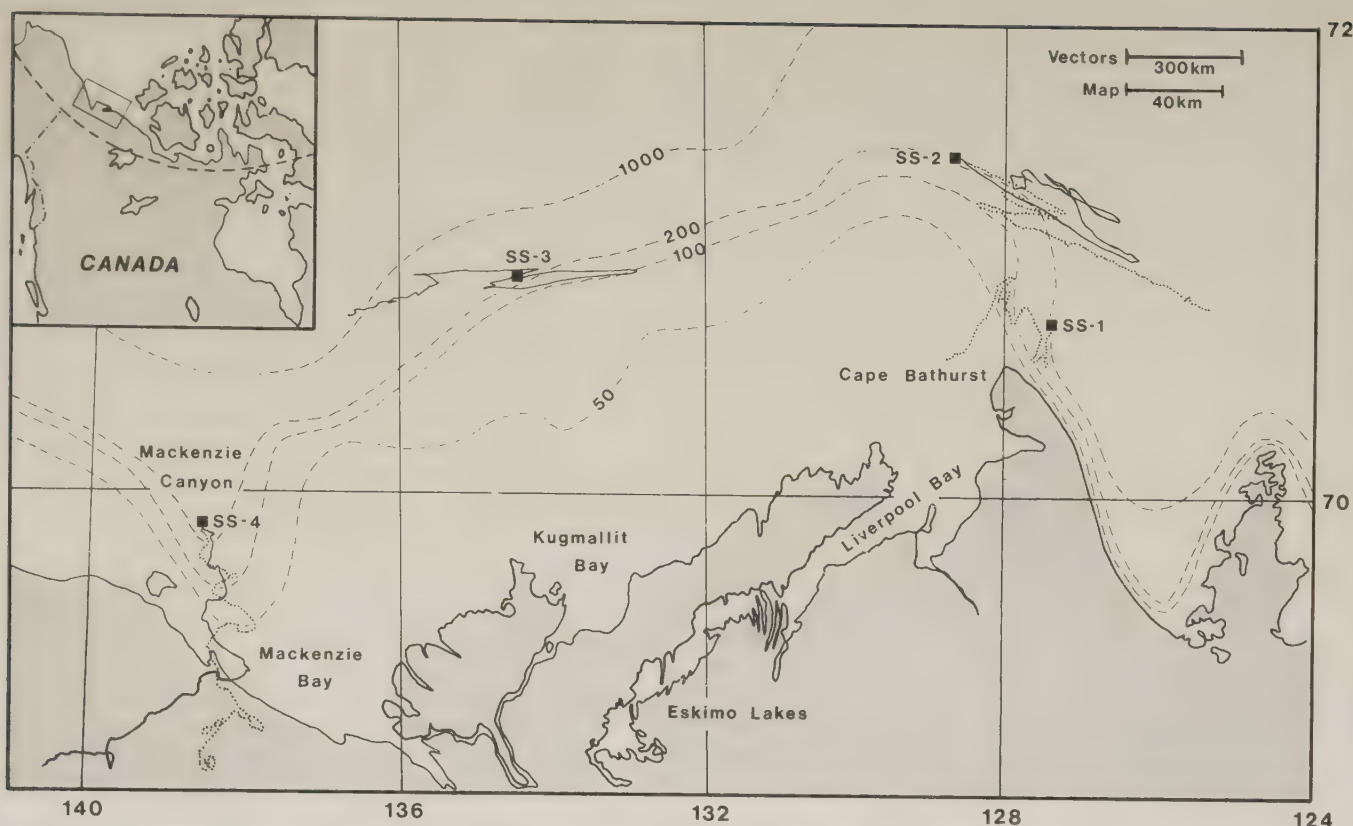


FIG. 1. Eastern Beaufort Sea, showing the location of sediment traps SS1, SS2, SS3, and SS4. Progressive current vectors are plotted on the map, with starting locations at the trap sites. Current meter data are unavailable for SS3 (133 m) and SS1 (35 m) due to instrument failure. (—, data from 35 m; ···, data from 120–150 m). The map scale varies with latitude. The scale provided is approximate for 70°N, 132°W. The vector scale is constant.

TABLE 1. Locations, water depths, and deployment depths of sequential trap deployment.

	Trap			
	SS1	SS2	SS3	SS4
Type	Parflux 6	S ³ T	Parflux 6	S ³ T
Latitude (N)	70°45.8'	71°27.3'	70°57.2'	69°51.0'
Longitude (W)	127°23.6'	128°39.8'	134°23.9'	138°29.6'
Water depth (m)	200	170	183	200
Deployment depth (m)	145	115	128	145
Salinity range	32.8 to 33.8	32.6 to 33.2	32.9 to 34.0	32.8 to 34.6
Temperature range (°C)	−1.4 to −0.9	−1.5 to −1.3	−1.4 to −0.8	−1.3 to −0.2

and 11 March 1988 at SS1 and SS3. Ice cover to the end of December 1987 was estimated from satellite imagery. NOAA-9 and -10 scanning multichannel microwave radiometer data were processed to indicate percentage ice cover within a 90 × 90 km box centered on each sediment trap location (Borstad et al. 1988).

The collection cups were filled prior to deployment with filtered (0.45 µm) seawater taken from the depth at which the traps were to be moored, with 2 g of sodium chloride added to each container to enhance internal trap density. Mercuric chloride (200 mg) was added as a preservative. On retrieval, collection cup contents were returned by air to the Institute of Ocean Sciences. Samples were kept cool during transit and refrigerated at 4°C until analyzed. Samples were screened through a 500-µm mesh, and the data reported here are derived from animals retained on the screen. Representative specimens of each taxon were preserved in 5% formaldehyde for later con-

firmation of identity. In some samples, preservation was poor, with the result that some or all taxa could not be sorted and counted (Table 2). Samples are designated by trap number - cup number (e.g. SS1-3 = trap SS1 sample 3).

To facilitate comparison among traps, quantitative data for the abundant taxa, copepods and pteropods, are normalized for area of trap and length of time of open deployment (number per square metre per day). The numbers and types of animals retained in sediment traps cannot be directly interpreted in terms of flux. Composition of the entrapped faunal communities may be compared among traps, but animals are differentially attracted or repelled by traps (Harbison and Gilmer 1986; Lee et al. 1988; Michaels et al. 1990), and differences in trap design may introduce bias in any quantitative analysis.

Agglomerative hierarchical cluster analysis, with complete linkage, was performed on the log-transformed ($\ln(x + 1)$) abundance data (Burd et al. 1990), with abundance expressed

as number per square metre per day. All taxa were included in this analysis. Normalized Euclidean distance between samples was used as the clustering metric, with a distance of 1.75 chosen as the level at which to classify samples into groups. Computations were performed with SYSTAT statistical software (Wilkinson 1990).

Results and Discussion

Currents and Ice Cover

Our traps, moored between 115 and 145 m in the water column, were located within the Arctic Surface Water. The base of this water mass is typically 200 m in this area (Carmack et al. 1989) and forms the boundary between waters of Pacific and Atlantic origin (Aagaard et al. 1985). Offshore of the Beaufort shelf, the motion in this water mass is dominated by the Beaufort Sea Gyre centered approximately at 77°N, 145°W (Thorndike and Colony 1982). This motion is evident in a weak westward current which makes its presence felt in the general westward drift of ice with mean speeds of the order of $2\text{--}4\text{ cm}\cdot\text{s}^{-1}$ (Giovando and Herlinveaux 1981). In contrast, the average subsurface motion along the slope, deeper than about 50 m, is eastward. Here, transport is organized in a relatively strong boundary current, the Beaufort Undercurrent, which has speeds of the order of $10\text{ cm}\cdot\text{s}^{-1}$ (Aagaard 1989). The water in this boundary current derives from varied sources which include inflow from the Pacific via the Bering Sea, shelf drainage of brine-enhanced water, and water from the basin interior (Macdonald et al. 1989). Onshore or offshore flow across the shelf break is influenced by upwelling, brine drainage, estuarine circulation, and flow instabilities; at present these processes are not sufficiently well understood to rank their importance.

During the collection period, monthly mean current speeds in the upper 35 m ranged from 5 to $20\text{ cm}\cdot\text{s}^{-1}$, with strongest flows occurring during the ice-free months (McCullough et al. 1988). Progressive current vectors show that currents were typically aligned with the bathymetry at SS2 and SS3 (Fig. 1). Persistent up-canyon flow was evident at station SS4, in Mackenzie Canyon, while at SS1 the deep currents were parallel to the bathymetry during the first part of the deployment, with a shift towards the shelf later. Temperature and salinity records from the current meters show that the sediment traps were typically situated within the upper halocline of the Arctic Ocean (Table 1). At SS4, within Mackenzie Canyon, two upwelling events in October and November brought water of Atlantic layer properties to the level of the trap.

In 1987, all of the mooring sites were in open water until the end of October. By 4–6 November, all sites appeared to be covered with new ice (>90%), although a confirming satellite image for SS1 was not obtained until 14 November. This would be considered a late freeze-up (Borstad et al. 1988). The satellite imagery collected well into December indicated that the sites remained fully covered, and all traps were recovered through solid ice cover at the end of March 1988.

Distribution of Taxa in Traps

Four species of calanoid copepods (*Euchaeta glacialis*, *Calanus glacialis*, *C. hyperboreus*, and *Metridia longa*) and the pteropod *Spiratella helicina* were the only animals retained in large numbers (Table 2). Other organisms collected included two species of hyperiid amphipod (*Hyperia medusarum* and *Themisto libellula*), two species of ostracod (*Conchoecia*

borealis maxima and *Philomedes globosa*) and, in poor condition, unidentified polychaete larvae of several species and unidentified isopods (Table 2). SS4 had the fewest species collected (7), while SS3 had the greatest number (13). Sample SS1-7 contained no animals. The copepods, ostracods, and pteropods collected are well-known inhabitants of the upper 200–300 m of the Arctic Ocean and adjacent seas in summer and deeper waters in winter (Johnson 1963; Grainger 1965). While one of the amphipods, *H. medusarum*, is generally restricted to coastal waters (Grainger 1965), the other, *T. libellula*, is known from both the central Arctic Ocean and margins (E. H. Grainger, Arctic Biological Station, P.O. Box 400, Ste-Anne-de-Bellevue, Que. H9X 3R4, pers. comm.). None of our taxa are typical of fauna associated with sea ice (Carey 1985) or benthos (Stewart et al. 1985; Snow et al. 1987; Wacasey 1975).

We infer from this composition of the zooplankton that the traps were located within the domain of Canada Basin water. This is in keeping with our measurements of currents parallel to or onto the shelf (Fig. 1). Further, Macdonald and Carmack (1991) have shown that due to ice topography the shelf waters become sharply divided in winter: freshwater from the Mackenzie River is contained close to shore in depths of less than 20 m, while the central portion of the shelf becomes decoupled from the nearshore and is subject to brine enhancement due to sea ice formation. All of these observations support the notion that the outer shelf tends to come under the influence of offshore waters during winter.

Cluster analysis of species composition and abundance (Fig. 2) illustrates the impact of the onset of ice cover at the beginning of November, during the latter part of the sample period 4 (19 October – 4 November), with the grouping of samples from SS2, SS3, and SS4 after this event. This grouping was the direct result of the appearance of pteropods in the samples (Fig. 3). Prior to ice cover, samples at these sites displayed considerable variability, although there are strong affinities between SS2-1, -2, -3 (31 August – 18 October), SS3-3 (3–19 October), SS4-2, and -3 (19 September – 19 October). The overall pattern at SS1 differs from the other locations. We conclude that this trap, at the entrance to Amundsen Gulf, lay outside the influence of the Beaufort Gyre and Beaufort Undercurrent which impinged on the other sites during this deployment. The current meter data indicate that water at depth at this trap site originated from Amundsen Gulf, to the south and east.

Details of Copepod and Pteropod Distributions

The total number of copepods in all traps (Fig. 3) was highest early in the sampling program ($100\text{--}375\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), declined to low numbers through midwinter ($\leq 65\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), and then increased to moderate numbers by March, where collections continued at SS1 and SS3 ($130\text{--}180\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). At SS2, about twice as many copepods ($325\text{--}375\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) were collected in autumn (SS2-1 to -3) compared with the other traps, and these high numbers continued later (into sample period 6) than at other sites. Although relatively few copepods were collected at SS4 in autumn and late winter, from late November into January (SS4-6 to -9), numbers were relatively high ($55\text{--}65\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). The increase in numbers seen at SS1 in early spring (starting at SS1-10) was repeated at SS3, and the similarity between these sites at this time is shown clearly in the cluster analysis (Fig. 2).

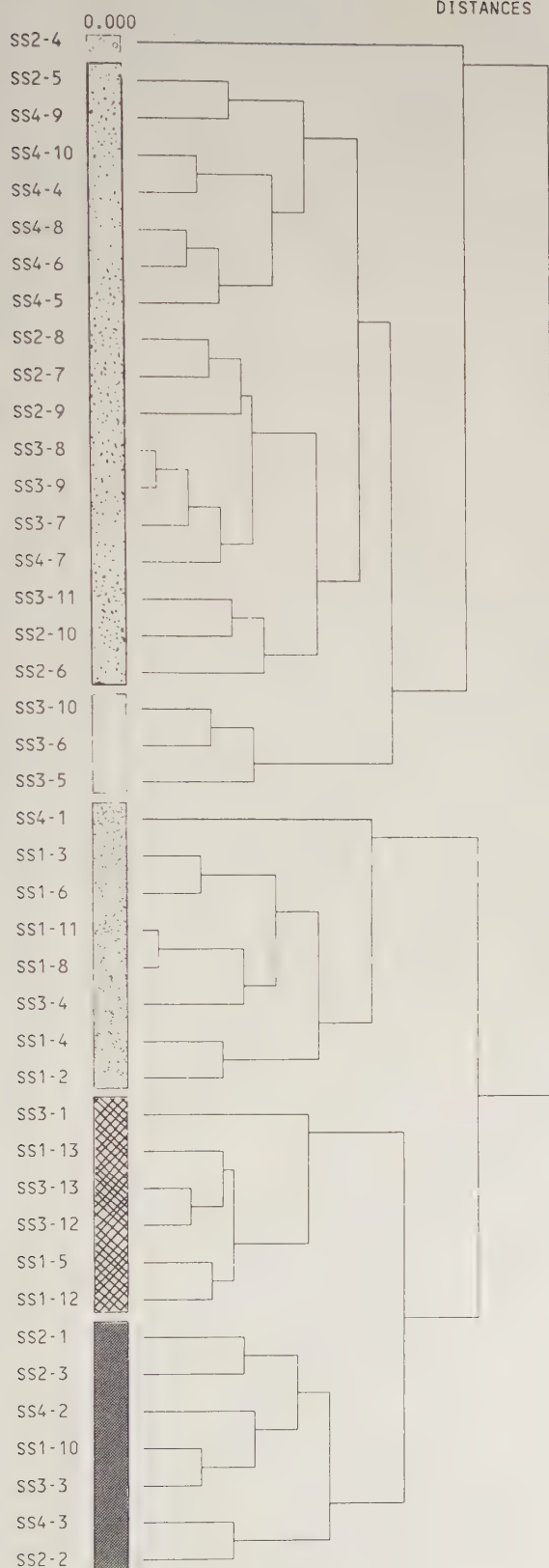
In contrast, the appearance of *S. helicina* in our samples coincided with the onset of ice cover (Fig. 3). These pteropods were collected only sporadically and in low numbers

TABLE 2. Number of specimens of each taxon retained in sediment trap samples. Where animals were not counted due to condition, this is indicated by n/c + = high concentration and n/c - = low concentration. Copepod stages are indicated (e.g. F = adult female; CIV f + m = stge IV, mixed sexes; us. = unstaged). Sample start dates are shown at the top.

	Sample No.												
	1 Sept. 1	17 Sept. 2	3 Oct. 3	19 Oct. 4	4 Nov. 5	20 Nov. 6	6 Dec. 7	22 Dec. 8	7 Jan. 9	23 Jan. 10	8 Feb. 11	24 Feb. 12	11 Mar. 13
SS1													
<i>Calanus hyperboreus</i> (F)					63					121		181	195
<i>Calanus glacialis</i> (M)												22	
<i>Euchaeta glacialis</i> (F)		297		68	77								
<i>Euchaeta glacialis</i> (us.)								1			n/c -	109	95
<i>Metridia longa</i> (F)				22	255			56		492	84	184	1147
<i>Metridia longa</i> (CIV f + m)												25	22
Calanoid (unspeciated)	n/c +			n/c -									
Isopod		4			3	1				3		3	4
<i>Hyperia medusarum</i>										3		1	
<i>Spiratella helicina</i>			31										
SS2													
<i>Calanus hyperboreus</i> (F)	11	33	3	4						2			
<i>Calanus glacialis</i> (M)	8		2	25									
<i>Calanus glacialis</i> (CIV)				13			1	3	2				
<i>Calanus glacialis</i> (us.)					2								
<i>Euchaeta glacialis</i> (us.)						6				4			
<i>Metridia longa</i> (F)	152	144	94	148		16	10	6	9	21			
<i>Metridia longa</i> (CIV f + m)						36	13	6	7				
<i>Metridia longa</i> (us.)					98								
Isopod		3		4	5		3	3					
<i>Hyperia medusarum</i>		1		1	1		1		2	2			
<i>Themisto libellula</i>			2										
<i>Polychaete larvae</i>				1									
<i>Spiratella helicina</i>			2	108	94	66	5	10	12	10			
SS3													
<i>Calanus hyperboreus</i> (F)			136	19			1	3	4	2	19	71	79
<i>Calanus glacialis</i> (M)			4	18				13	8	6			
<i>Calanus glacialis</i> (CIV)							1						
<i>Calanus glacialis</i> (us.)						7							
<i>Euchaeta glacialis</i> (F)	665												
<i>Euchaeta glacialis</i> (CV f + m)													238
<i>Euchaeta glacialis</i> (us.)	4			22			1	5	8	21	66	100	
<i>Metridia longa</i> (F)	652		114	80		12	136	228	169	38	71	409	764
<i>Metridia longa</i> (CIV f + m)									6				
Calanoid (unspeciated)		n/c +			33								
Isopod	9		1	1		1	1	1		5			2
<i>Hyperia medusarum</i>			3		4		1	1	1	1			
<i>Themisto libellula</i>						2	1			1			
<i>Polychaete larvae</i>							1	1					
<i>Spiratella helicina</i>	8		n/c -	17	724	380	132	204	246	611	210	28	9
Eggs (?)										n/c +			
<i>Conchoecia borealis maxima</i>									1				
<i>Philomedes globosa</i>								1					
SS4													
<i>Calanus hyperboreus</i> (F)		6	15	2	3	2		2		4			
<i>Calanus glacialis</i> (M)		1							3	1			
<i>Metridia longa</i> (F)		59	34	6	9	27	10	32	10	8			
<i>Metridia longa</i> (CIV f + m)							20						
Calanoid (unspeciated)	35												
Isopod	1		12	1	14	8	1	4	7	1			
<i>Hyperia medusarum</i>	1	3		1					1	3			
<i>Spiratella helicina</i>			26	51	20	29	10	29	68	60			

TREE DIAGRAM

DISTANCES



5.000

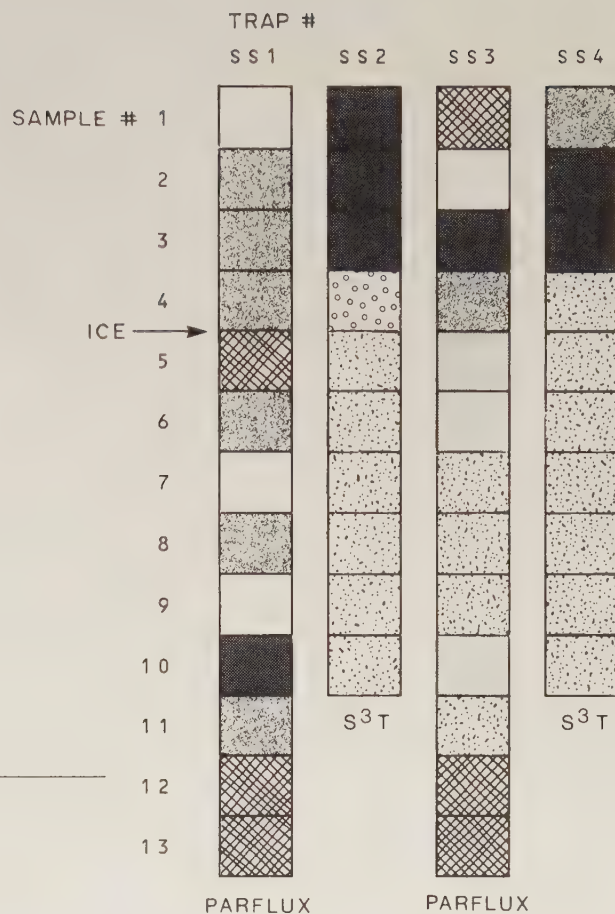
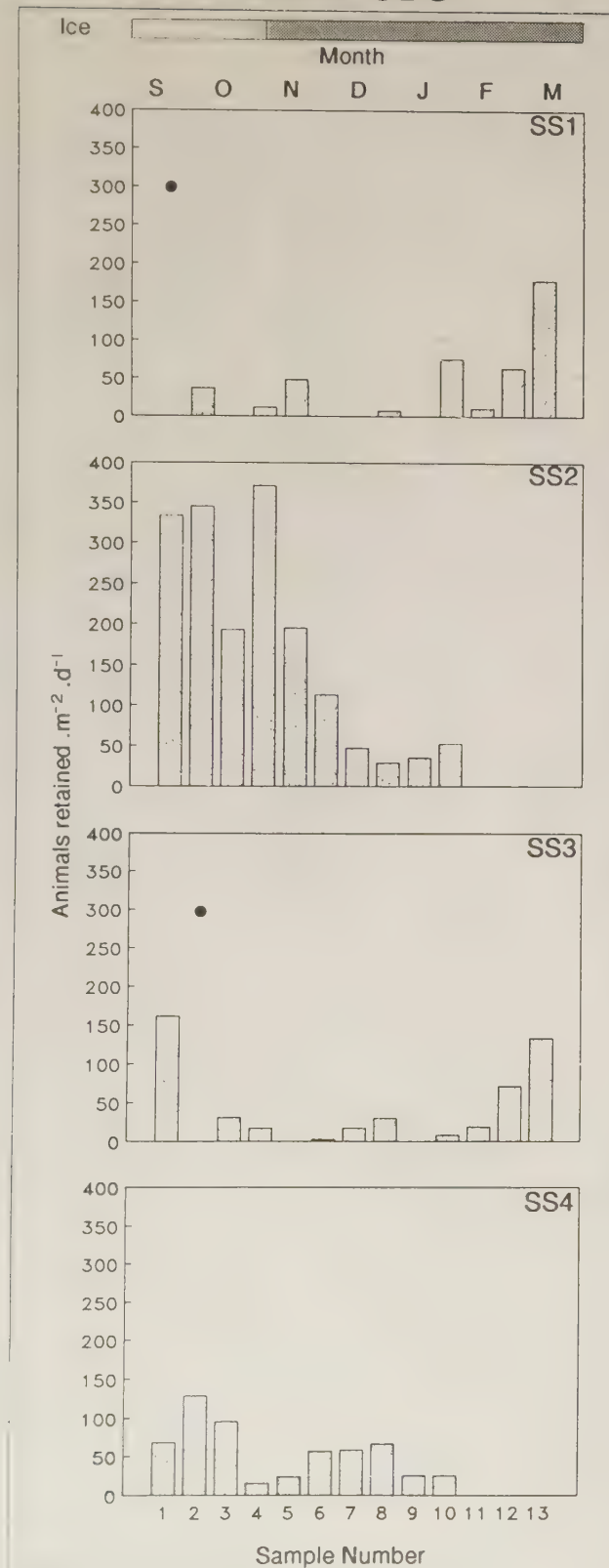


FIG. 2. Tree diagram of cluster analysis of sediment trap species abundance. Upper right: cluster membership by trap and sample number. Blank: sample not included in analysis.

COPEPODS



PTEROPODS

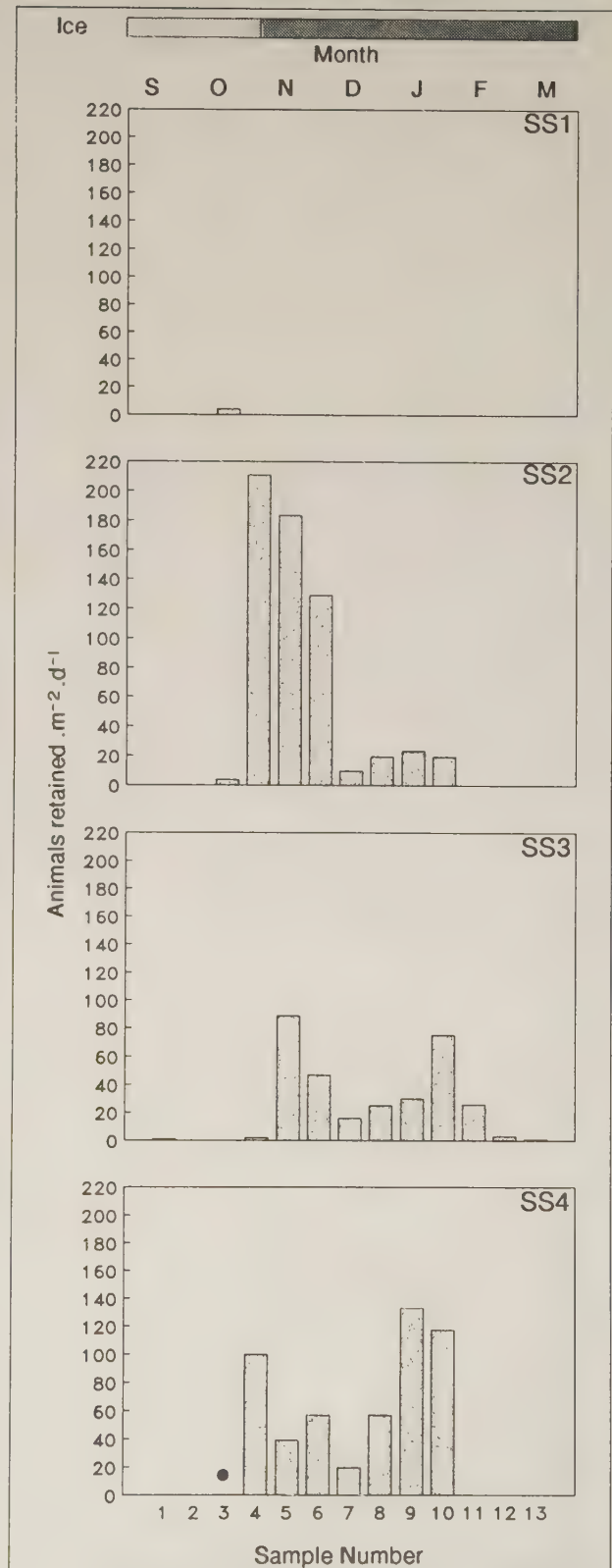


FIG. 3. Left: Total copepods retained by sample period. Circles indicate large numbers of uncountable, decayed copepods from SS1-1 and SS3-2. Right: Total pteropods retained by sample period. The circle shows small numbers of pteropods that could not be counted for SS4-3. Top: Bars show percentage ice cover. Light shading represents <10%; dark shading represents >90% (assumed for January–March when traps were recovered through solid ice). The months during the sampling period are indicated by their initial letters.

(<10·m⁻²·d⁻¹) during open water at SS1, SS3, and SS4. When ice appeared at the beginning of November, pteropods immediately increased to over 200·m⁻²·d⁻¹ at SS2 and about 100·m⁻²·d⁻¹ at SS3 and SS4. At SS2 the numbers gradually declined through to December (SS2-6), after which they varied between 10 and 25·m⁻²·d⁻¹ for the remainder of the winter. At SS3 and SS4, numbers were variable throughout the winter, ranging from 2 (SS3-4) to 132·m⁻²·d⁻¹ (SS4-9). In late February and March, after SS2 and SS4 sampling ceased, very few specimens were collected at SS3. At SS1, pteropods were collected only once, at SS1-3 in mid-October, with fewer than 3·m⁻²·d⁻¹. In the central Arctic Ocean, Kobayashi (1974) found *S. helicina* to aggregate in the upper 50 m during September, remain concentrated in the upper 100 m during winter, but disperse throughout the water column to 1200 m during summer. We infer therefore that the abrupt appearance of pteropods in our samples coincided with the upward consolidation of the population in late autumn, which may have been triggered by the onset of local ice cover.

The most abundant species of copepod at all sites was *M. longa* (Table 2). *C. hyperboreus* and *E. glacialis*, though rare in the middle of winter, augmented concentrations of *M. longa* in late fall, early winter and spring. *Calanus glacialis* occurred sporadically, usually in low numbers at all sites. The majority of copepod species found off the continental shelf in the Arctic Ocean are not abundant and reside at depths greater than 200 m (Johnson 1963). In contrast, the four copepod species we collected reside primarily above 200 m (Johnson 1963; Grainger 1975) and were the most abundant species in Johnson's (1963) collections in the high polar basin, both in winter and summer. He found, as we did, that *M. longa* was the most numerically abundant. Calanoid copepods also dominated samples collected in a sediment trap off Axel Heiberg Island (Hargrave et al. 1989), approximately 1100 km northeast of SS2. Although there were differences in species composition and abundance, *C. hyperboreus* and *M. longa* were the most abundant overall. In contrast, the winter observations on the Beaufort Shelf by Erickson et al. (1983) showed that *Pseudocalanus* sp. dominated the fauna almost exclusively, with occasional specimens of *Metridia* sp., *Calanus* sp., and cyclopoid copepods collected in February. Nearer shore, animals of typically freshwater origin (F. F. Slaney and Co. Ltd. 1974) or low-salinity environments (Evans and Grainger 1980) were dominant.

Calanus hyperboreus collected from ice islands in the upper 200 m of the central Arctic Ocean were almost exclusively adult females from November to May (Dawson 1978; Rudyakov 1983), since adult males and copepodites winter at depths of 300–600 m during this time. All *C. hyperboreus* specimens in our samples were also adult females, as were the majority of specimens of *E. glacialis* and *M. longa* (Table 2). However, low numbers of copepodite stage CIV *M. longa* did appear sporadically at all sites during the winter. With our traps sampling at a fixed depth, we are unable to determine whether the typical, seasonal downward migration of copepodites and adult males occurred prior to our sampling, or if adult females of these species were distributed farther onto the shelf than the other stages. We note that, in contrast with the other species, the adult *C. glacialis* that we collected were male. A few CV specimens of this species were also found.

Interactions between Animals and Traps

The low diversity of species in our samples, compared with typical net collections in this area (Johnson 1963; Grainger

1965), is partly due to selectivity of the traps with respect to species retained and partly due to our sieving method (e.g. limitation to animals larger than 500 µm). Except in a few cases where very large numbers were collected, the condition of the animals in our traps was good. As virtually all empty carapaces found were from adult copepods, crustacean molts did not contribute significantly to particulate flux. This is in contrast with spring in the inshore area of the western Beaufort Sea, where sediment trap material collected from April to June contained a large number of molts (Carey 1987). All pteropods examined closely still had body contents within their shells, with no evident decomposition. Collection of pteropods is selectively enhanced in sediment traps (Harbison and Gilmer 1986). Their escape mechanism on disturbance, such as contact with a mooring cable, is to sink immediately and rapidly, and entry into sediment traps is presumably irreversible for them. If the trapping of large numbers coincided with fall and winter aggregation in the upper 100 m, this mechanism would account for collection in our sediment traps at 115–145 m.

While the absolute abundance of animals in the larger diameter traps, SS1 and SS3, was greater than in traps SS2 and SS4 (Table 2), cluster analysis (Fig. 2), normalized to trap area, shows that environmental effects were more pronounced than effects of trap design. We did not determine the relative catch efficiency of the Parflux and S³T traps.

Conclusions

The physical and behavioral dynamics affecting the distribution of zooplankton along the Beaufort shelf break varied for different groups during winter. The pattern of copepod species and stages collected reflects the interaction of traps sampling at a fixed depth with the seasonal variation in vertical distribution of this group. In contrast, the distribution of pteropods reflects a close coupling with the presence of ice cover. However, we cannot determine whether ice cover controlled vertical distribution or was coincidentally associated with onshelf advection of pteropods. Because the traps were devoid of animals restricted to the continental shelf, we infer that traps SS2, SS3, and SS4 were located in the domain of Canada Basin water. The differences in the relative distribution of copepods, absence of pteropods, and fewer number of species collected at SS1 indicate that community dynamics here differed from the other trap sites. Current meter data reveal that the source water at this site was from Amundsen Gulf, to the south and east.

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Influence of Tributary Spatial Position on the Structure of Warmwater Fish Communities

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We found a significant and positive relationship between fish species richness and four measures of stream size (drainage area, stream order, link magnitude, and downstream link) in three Illinois drainage basins. Downstream link (incorporating both stream size and size of stream at the next downstream confluence) explained the greatest portion of the variance. This suggests that downstream processes significantly influence the structure of fish communities inhabiting warmwater streams. Significantly higher numbers of fish species were collected from tributary streams (<259 km² drainage area) located lower in a drainage network and connected to a main channel system than from similarly sized streams located in the headwaters of a drainage network. The difference in species richness among station treatments was not due to a difference in the number of individuals collected among treatments. We were unable to accept or reject the hypothesis that differences in fish species richness were due to differences in physical habitat. The immigration–extinction hypothesis appears to provide a plausible explanation for the observed pattern in fish community structure within a drainage. The location of a stream channel within a network may provide a general template for fish community structure in warmwater drainages by regulating potential species richness.

Une corrélation significative et positive a été observée entre la diversité des espèces de poissons et quatre variables se rapportant à la taille des cours d'eau (aire drainée, ordre du cours d'eau, grandeur du segment d'un confluent à l'autre et segment d'aval) dans trois bassins de drainage de l'Illinois. Le segment d'aval (paramètre intégrant la taille du cours d'eau et sa taille au prochain confluent, en aval) était responsable de la plus grande partie de la variance. Cela laisse entendre que les processus qui se déroulent en aval ont une influence déterminante sur la structure des communautés de poissons vivant dans des cours d'eau tempérés. Le nombre d'espèces dans les tributaires (aire drainée de moins de 259 km²) situés plus en aval dans le réseau hydrographique et se jetant dans un segment principal du réseau était nettement supérieur à celui observé dans les cours d'eau de taille semblable mais situés à la tête des réseaux hydrographiques. Les différences de diversité observées entre les stations ne sont pas attribuables aux différences entre les nombres d'individus recueillis aux diverses stations. Les résultats n'infirment ni ne corroborent l'hypothèse suivant laquelle la diversité des espèces serait liée aux caractéristiques physiques de l'habitat. L'hypothèse de l'immigration–extinction semble fournir une explication plausible de la configuration observée de la structure des communautés dans des réseaux hydrographiques. La position des segments dans les réseaux hydrographiques tempérés peut jouer un rôle dans la détermination de la structure des communautés de poissons vu son effet régulateur sur la diversité des espèces.

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Numerous process-oriented studies conducted on small watersheds (Meyer et al. 1988) have led to the development of lotic paradigms that emphasize the linkage between downstream community structure and function and upstream processes (Cummins 1974; Cummins et al. 1984; Vannote et al. 1980; Minshall et al. 1985; Minshall 1988; Newbold et al. 1981, 1983; Ward and Stanford 1983). Empirical evidence has also demonstrated that basin characteristics (e.g. riparian vegetation, urbanization, slope, geologic age) influence processing rates and the distribution and abundance of lotic organisms in stream channels (e.g. Wiley et al. 1990; Covich 1988; Naiman et al. 1987; Matthews 1985). Large-scale basin investigations suggest that fish species richness generally increases with increasing drainage area in low- to midorder streams (Larimore and Smith 1963; Sheldon 1968; Horwitz 1978). This pattern appears to hold across a wide range of latitudes (Lake 1982; Welcomme 1985; Angermeier and Schlos-

ser 1989), although it is not yet clear whether it is a result of increasing habitat diversity, differential immigration and extinction rates (Power et al. 1988), or simply a sampling phenomenon because larger areas generally support more individuals (sensu Angermeier and Schlosser 1989). This relationship between fish species richness and stream drainage area has led to the prediction that streams of similar drainage areas within a region would have comparable species diversity (e.g. Minshall et al. 1985). Further, the upstream–downstream emphasis has promoted the idea that lotic ecosystems are linear, rather than being composed of a complex hierarchical network of individual reaches.

Recent studies, however, have documented limitations associated with the use of a single, generalized conceptual framework that constrains structural patterns and functional processes to a simple upstream–downstream pathway (Naiman et al. 1988; Junk et al. 1989; Wiley et al. 1990; Sparks et al. 1990).

We suggest that because of the emphasis on a unidirectional flow of energy and materials, downstream influences on upstream structural patterns may have been underestimated and/or overlooked. For instance, recovery rates following natural disturbances may vary with spatial location in a system, thus influencing community structure (Connell 1978; Resh et al. 1988; Schlosser 1985; Paine and Levin 1981; Wilbur 1987). With few exceptions (e.g. Fausch et al. 1984; Gorman 1986), the influence of a stream's position in a drainage network and the influence of downstream attributes on upstream community structure have received limited attention.

In this paper we investigate the influence of channel position in a drainage network on the structure of fish communities. Specifically, we hypothesize that fish communities in tributaries to main channels have greater species diversity than communities in streams of similar size located in the headwaters of a basin. In addition, we speculate on potential mechanisms responsible for the observed differences in species richness.

Methods

Geomorphic Metrics

Traditionally, stream order (Horton 1945; Strahler 1957) and drainage area have been used to classify lotic systems and as surrogates for stream size (e.g. Larimore and Smith 1963; Horwitz 1978; Vannote et al. 1980; Lake 1982). Tributaries originating at the source are designated as order 1 (Fig. 1a). Order is incrementally increased below the confluence of two streams of the same order. The junction of two streams of unequal order does not increase stream order; rather, the downstream reach retains the highest value of the two merging streams (Fig. 1a).

While seldom used in ecological investigations (but see Bruns et al. 1982), link magnitude (Scheidegger 1965) was developed to account for the addition of tributaries of order $x - n$ (where

$n \geq 1$) to a section of order x . Such lesser tributaries increase discharge and the magnitude of associated hydrological variables (e.g. wetted perimeter, depth), yet have no influence on stream order. The magnitude of a link is the number of first-order segments (*sensu* Horton 1945) upstream of a given point on a channel (Fig. 1b).

The magnitude of stream order, drainage area, or link magnitude is entirely dependent upon upstream characteristics. These metrics, however, do not describe the spatial location of a smaller stream within a drainage network. For instance, none of these metrics describes the influence of spatial juxtaposition of tributary streams to much larger, but downstream, system reaches with greater water volume and more varied habitat types. Gorman (1986) referred to such systems as adventitious streams. To our knowledge, no single geomorphological variable describes the relation of a given stream reach to upstream and downstream influences within a drainage network. We therefore adopted the downstream link (D-link) as a crude index of the spatial location of a stream within a drainage network.

The D-link at any point in a drainage network is the magnitude of the link of the channel below the next downstream confluence (Fig. 1b). The D-link metric can be calculated from standard topographic maps and has the advantage that tributaries located in the headwaters of a drainage network have lower D-link values than do similarly sized (equivalent drainage area, stream order, and link magnitude) streams located farther downstream in the drainage network. For instance, stations A and B (Fig. 1b) have the same drainage area, stream order, and link magnitude, but the D-link values at the two stations are 2 and 15, respectively.

Like all environmental metrics of habitat condition, the D-link is not without limitations. The most significant is the effect of a small tributary that is located immediately downstream of a point of interest, but immediately upstream

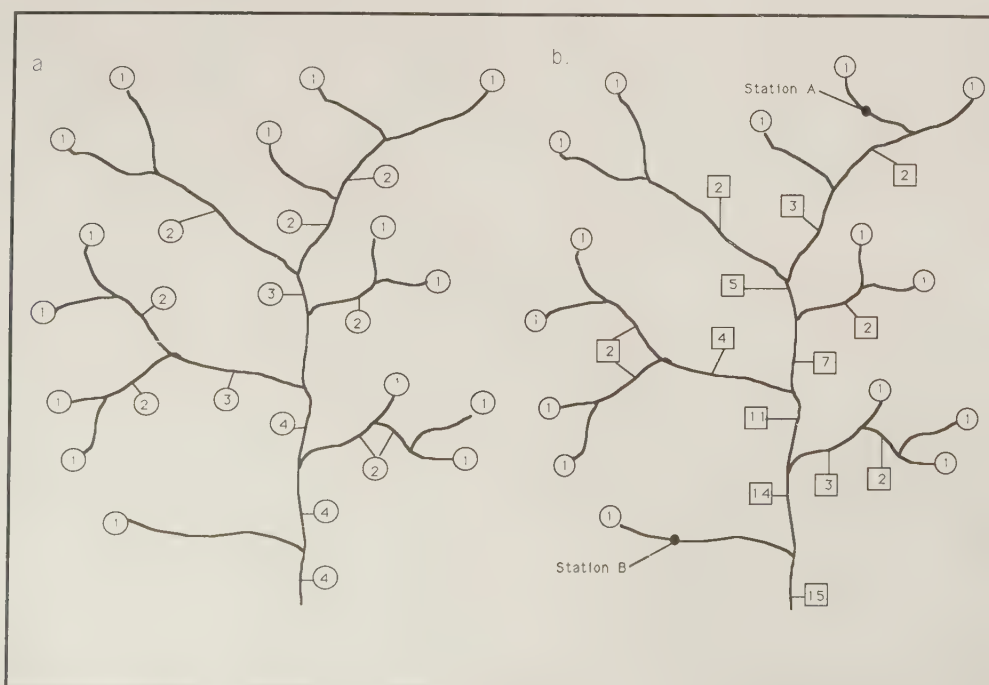


FIG. 1. Example of the determination of (a) stream order and (b) link magnitude. To determine the downstream link (D-link), select a location within the "b" network. The D-link value will be the magnitude of the link at the next downstream confluence. See text for details.

from a substantially larger channel. This situation was encountered only once in our study. In this instance, we elected to bypass the most immediate downstream junction (link magnitude of 1) because of the probable influences of the proximate and larger system. Other limitations are undoubtedly associated with the use of this variable and we do not expect that any single index will sufficiently account for the complex interactions and processes responsible for the structuring of biological communities.

Study Area

We collected fish distribution data from the Salt Fork and Middle Fork of the Vermilion River in east-central Illinois, USA (Fig. 2). These basins are spatially juxtaposed and encompass a combined area of 2313 km². Row crop agriculture accounts for more than 90% of the land use (Osborne and Wiley 1988). Forested riparian vegetation is generally restricted to mature gallery forests in the lower portion of the main stem of both rivers (Wiley et al. 1990). As is characteristic of this region, most low-order streams were channelized to facilitate drainage between 1890 and 1920.

Twenty-two fish sampling stations were established in streams in the Vermilion River basin (Fig. 2) and assigned to one of three station treatments that reflected both stream size and general spatial location: headwater tributary (HT), main

channel tributary (MT), and main channel (MC) streams. In each of the Salt Fork and Middle Fork subbasins, a distinct network main channel roughly corresponded to a fifth-order or greater stream. MC stations had drainage areas greater than 250 km² and were categorized as order 5 or greater. Each sub-basin was divided into an upper and lower portion on the basis of the order of the stream. Tributaries with drainage areas less than 259 km² were divided into MT and HT streams based on their relative location within the drainage network. HT streams flowed into other HT streams or joined to form a main channel and were located in the upper half of a subbasin. MT streams flowed into MC streams and were located in the lower half of a subbasin. Stream reaches outside of these categories were not sampled and are not included in the present study. Seven sampling stations were located in HT, seven in MT, and eight in MC streams of the Salt and Middle Fork subbasins (Fig. 2).

A majority of smaller streams in this region have historically been channelized or hydraulically modified. To minimize the potential influence of channelization on results, five of the seven MT and six of the seven HT sampling stations were located in reaches of streams that had been channelized generally more than 60 yr ago. This ratio of hydraulically modified/unmodified sampling stations was roughly equivalent to the actual ratio of modified/unmodified streams within the Vermilion River drainage. The distance of MT stations to the nearest downstream main channel ranged from 3.2 to 20.2 km (Fig. 2).

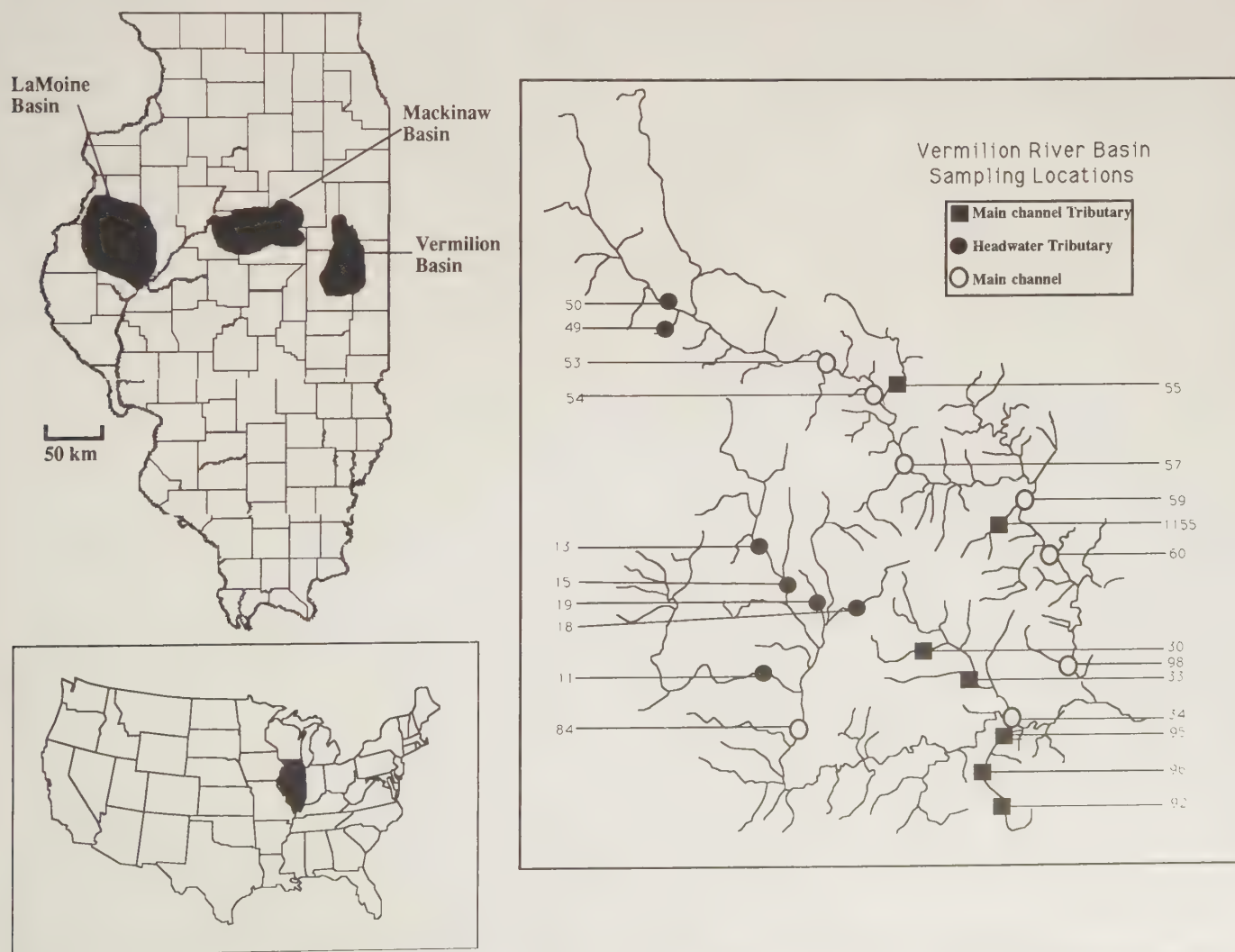


FIG. 2. Fish sampling locations in the Vermilion basin and the location of the Mackinaw, LaMoine, and Vermilion drainages in Illinois.

TABLE 1. Sampling stations, total number of fish species collected categorized by station treatment (drainage network location and stream size) within the Vermilion, Mackinaw, and LaMoine basins, and associated stream order. Station categories correspond to those described in the text. Station numbers for the Mackinaw and LaMoine basins correspond to those used by Day et al. (1990, 1992).

Basin	Headwater tributary (HT)			Main channel tributary (MT)			Main channel (MC)		
	Station	No. of spp.	Order	Station	No. of spp.	Order	Station	No. of spp.	Order
LaMoine	DGL-07	9	4	DGIA-03	23	3	DG-01	20	6
	DGLD-01	11	3	DGD-01	17	3	DG-02	20	6
	DGLC-01	13	2	DGJ-04	12	3	DG-06	12	6
	DGL-08	12	4	DGJ-03	13	3	DG-07	21	6
	DGL-05	17	2						
Mackinaw	DKV-01	22	3	DKE-01	24	4	DK-19	32	6
	DKT-01	10	4	DKG-01	26	4	DK-04	28	6
	DKP-02	16	4	DKJ-01	36	4	DK-15	31	6
	DKKB-01	22	4	DKB-01	24	2	DK-16	28	6
	DKKC-02	21	3						
Vermilion	11	12	4	33	28	4	34	33	5
	13	19	4	55	16	4	53	23	5
	15	15	3	92	17	2	54	23	5
	18	22	3	95	21	2	57	26	5
	19	14	1	96	23	2	59	28	6
	49	9	2	30	23	3	84	18	5
	50	15	4	1155	17	2	98	31	6
							60	27	6

Fish Sampling Procedures

Fish were collected from a representative 100- to 200-m reach at each station in the Vermilion River basin during October 1984 or 1985. Due to the range in stream sizes (e.g. width and depth) studied, it was necessary to use two sampling techniques. Smaller, low-order streams (i.e. HT and MT) were sampled with a 9.1-m electric seine (Bayley et al. 1989) powered by a portable AC generator (1500 W, 120 V). The seine was fished upstream through the reach by a minimum of three individuals, including the two seine operators. Stunned fish were collected in standard dip nets. In larger, deeper streams, fish were collected using a two-man, three-phase, boom-type, boat-mounted electrofisher powered by an AC generator (3000 W, 230 V). The unit was mounted on a 5.7-m shocking boat propelled by a 10-hp gas motor. Fish were preserved in 7% formalin and returned to the laboratory for identification and enumeration.

To minimize the possibility that any observed pattern in fish species richness was basin specific, we incorporated into our analyses species richness data reported by Day et al. (1990) from 13 stations in the Mackinaw River (drainage area 3013 km²) in central Illinois and data reported by Day et al. (1992) from 13 stations in the LaMoine River (drainage area 3522 km²) in west-central Illinois. With the exception of the modifications described below, we used only data from sampling stations that met our criteria for HT, MT, and MC streams. Because the drainage area of each station was unavailable, we categorized stations as HT sites on the basis of size (fourth order or less, originally determined from 7.5' topographic maps) and location in the upper portion of their respective drainage networks. Similarly, stations were categorized as MT on the basis of size (fourth order or less) and relative proximity to the main channel in the lower half of their respective basins (Table 1).

The data from the three drainage basins (i.e. Vermilion, Mackinaw, and LaMoine) represent collections that were made from midsummer through fall over a 4-yr period (Vermilion basin: 1984 and 1985; Mackinaw basin: 1987; LaMoine basin:

1988). Thus, the influence of either a single annual event or a basin-specific response on the overall spatial pattern in the data would be minimized, while the variance would be increased. Further, the structure of the fish communities should have been relatively stable, as communities in tributaries (HT and MT) were more likely to have been composed of resident populations during this period. For instance, this period is typically characterized by a relatively stable hydrological regime, fish populations should be minimally influenced by recruitment, and larger river species that enter the tributaries to spawn should have returned to the main channel areas.

We determined the order (sensu Strahler 1957), link magnitude (Scheidegger 1965), and downstream link at each sampling station in the Vermilion River basin using 7.5' U.S. Geological Survey (USGS) (1:24,000 scale). Drainage area for each sampling station was determined using the Illinois Geographic Information System according to methods described in Osborne and Wiley (1988). Maps used in calculating link magnitude and downstream link values of stations in the LaMoine and Mackinaw basins were on a scale an order of magnitude greater (1:250,000) than those used for the Vermilion River basin. Using larger scale maps undoubtedly resulted in lower link magnitude values for equivalently sized channels in the Vermilion River; therefore, we did not compare the magnitude of the geomorphic variables between basins. Instead, we assumed that map scale did not influence our ability to detect meaningful patterns in the distribution of species between basins.

Data Analysis

All statistical analyses were performed with SYSTAT® (Wilkinson 1988) using $\alpha = 0.05$. We initially tested for differences in fish species richness at sampling stations using a full factorial, two-way analysis of covariance (ANCOVA) with basins and station size/location (i.e. HT, MT, and MC) as the main effects and number of individuals (log transformed) as the covariate. We incorporated number of individuals into this

analysis because a preliminary investigation revealed a rather weak association ($R^2 = 0.09$, $F_{1,46} = 4.367$, $p = 0.042$) between species richness and the transformed number of individuals. Because the primary and secondary interaction terms in the initial ANCOVA were not significant, they were removed, so the final model consisted of only the two main effects and the covariate. Differences in species richness and total abundance due to the main effects of station treatment and drainage basin were ascertained using direct post hoc testing.

Similarity in species composition (presence-absence) among station treatments within each drainage basin was assessed using agglomerative, single linkage, hierarchical cluster analysis and percentage similarity as a distance index. The relationships between fish species richness and the geomorphologic variables stream order, link magnitude, drainage area, and downstream link were determined using simple linear regression models.

Information on physical habitat was obtained for several stations in the Vermilion and Mackinaw basins from previously published studies (Wiley et al. 1987; Osborne et al. 1987; Short 1987) and analyzed to determine if patterns in fish species richness among station treatments were related to differences in physical habitat. In the Vermilion basin, data on substrate size and composition, channel depth, and water flow, collected along transects at fish sampling stations, were used in calculating hydraulic diversity, mean substrate particle size, and coefficient of variation in depth. These data were analyzed for differences among station treatments using a one-way ANOVA. Similar analyses were performed on the average stream depth, water velocity, and instream cover data for stations in the Mackinaw basin. Information on channel gradient for the upper and lower half and the total length of each HT and MT stream was generated from hypsometry data obtained from the Illinois Streams Information System (ISIS) database at the Illinois Natural History Survey. Differences in the gradient of the total length, the upper half, and the lower half of HT stations within each drainage basin were compared with similar data from MT stations within each drainage basin using a two-way ANOVA. Because initial testing revealed no significant interactions between the two main effects (basin and tributary location) in any of the three analyses of gradient (total, upper half, and lower half), the final models only considered the main effects.

Discharge

To determine if differences existed in discharge variability among station treatments, historical (January 1, 1959, to September 1, 1987) daily discharge data were obtained from two USGS gauging stations in the Vermilion River basin. Discharge data from USGS station No. 03336900 (located downstream of stations 18 and 19 and referred to here as station DHT, drainage area 347 km²) were used to develop annual hydrographs representative of HT streams. While slightly larger than the criterion used for HT streams (i.e. 259 km²), we do not believe that this difference influenced our results. Discharge data from USGS gaging station No. 0333900 (referred to here as station DMC, drainage area 3341 km²), located near Danville, Illinois, were used as representative of flows in larger MC reaches. In addition to these USGS gauging stations, we obtained average daily discharge data from a calibrated, constant monitoring water level recorder located at station 95 (drainage area 57 km²; Fig. 2), an MT station. Data from station 95 were limited to the period April 20 to November 15, 1985, and September 1988–90. Basic statistics from these data were compared with

TABLE 2. Results of two-way ANCOVA on fish species richness with respect to drainage basin (Mackinaw, LaMoine, and Vermilion), station treatment (HT, MT, and MC), and the transformed (natural logarithm) number of individuals as the covariate, and post hoc contrasts for station treatments and drainage basins.

Source	SS	df	MSQ	F-ratio	p
Drainage basin	545.60	2	272.80	13.529	0.00003
Station treatment	808.68	2	404.34	20.053	<0.00000
ln (Individuals)	67.58	1	67.58	3.351	0.07425
Error	846.88	42	20.16		

Post hoc contrasts for station treatments

MT > HT ($F_{1,39} = 15.13$, $p = 0.000$)

MT = MC ($F_{1,39} = 2.73$, $p = 0.106$)

HT < (MT + MC) ($F_{1,39} = 31.04$, $p = 0.000$)

Post hoc contrasts for drainage basins

LaMoine < Vermilion ($F_{1,39} = 10.04$, $p = 0.03$)

Vermilion < Mackinaw ($F_{1,39} = 5.67$, $p = 0.022$)

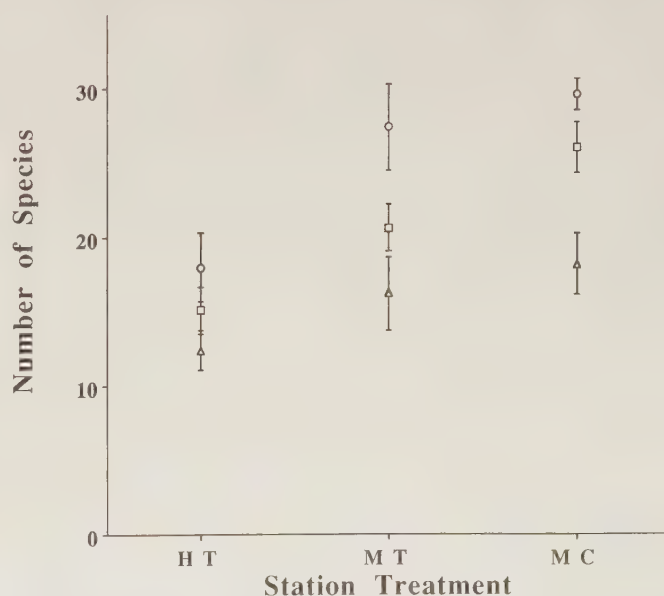


FIG. 3. Mean ($\pm$ SE) number of fish species collected at each station type in the LaMoine (triangles), Mackinaw (circles), and Vermilion (squares) basins. HT = headwater tributary stations, MT = main channel tributary stations, and MC = main channel stations.

data from stations DHT and DMC collected during comparable time periods.

A coefficient of variation of mean daily discharge ($CV(Q)$) for each year and station was calculated and used as an index of variability in annual discharge (see Horwitz 1978 for discussion). Average annual values for each year of the 29-yr period of record were compared using a paired *t*-test.

Results

A total of 50 fish species were collected from 22 sampling stations in the Vermilion River, with 54 and 43 fish species collected from 13 stations in the Mackinaw and LaMoine basins, respectively. In all three drainage basins, minimum species richness occurred at HT stations and ranged from nine taxa in the Vermilion and the LaMoine basins to a minimum of 10 in the Mackinaw River basin (Table 1). The maximum species richness in the Vermilion River basin was 33 and occurred at

TABLE 3. Groupings of fish sampling stations generated from hierarchical cluster analysis of fish presence-absence data. Codes in parentheses correspond to the treatment categories in Table 1. (a) Data from the Vermilion basin; (b) data from the Mackinaw basin (after Day et al. 1990); (c) data from the LaMoine basin (after Day et al. 1992).

	Group 1		Group 2		Group 3	
(a)	18 (HT)	1155 (MT)	95 (MT)	54 (MC)	59 (MC)	
	13 (HT)	15 (HT)	30 (MT)	57 (MC)	60 (MC)	
	11 (HT)		49 (HT)	53 (MC)	33 (MT)	
	19 (HT)		55 (MT)	50 (HT)	34 (MC)	
	84 (MC)		92 (MT)	96 (MT)	98 (MC)	
(b)	DKB-01 (MT)		DKJ-01 (MT)		DKT-01 (HT)	
	DK-19 (MC)		DKE-01 (MT)		DKP-02 (HT)	
	DK-15 (MC)		DKKC-02 (HT)		DKV-01 (HT)	
	DK-16 (MC)		DKKB-01 (HT)			
	DK-04 (MC)		DKG-01 (MT)			
(c)	DG-01 (MC)		DGL-7 (HT)			
	DG-07 (MC)		DGLD-01 (HT)			
	DGL-08 (HT)		DGIA-03 (MT)			
	DG-02 (MC)		DGJ-04 (MT)			
	DG-06 (MC)		DGL-05 (HT)			
			DGJ-03 (MT)			
			DGLC-01 (HT)			
			DGD-01 (MT)			

MC stations 34 (Table 1). In both the LaMoine and Mackinaw basins, maximum species richness values occurred at MT stations. Maximum species richness values were substantially higher, 36, in the Mackinaw basin compared with the 23 taxa collected from the LaMoine basin (Table 1).

Both station treatment (i.e. spatial location and size) and drainage basin had a significant influence on the number of fish species occurring within a stream reach (Table 2). No significant influence of the covariate, number of individuals, was found on species richness (Table 2). Among the tributary treatment categories, fish species richness was significantly greater at MT stations than at HT stations (Fig. 3; Table 2). There were no significant differences in the number of fish species collected between MC and MT stations across basins (Table 2; Fig. 3). Significantly fewer species were collected at HT stations than at MT and MC stations combined. Across basins, HT streams supported an average of 15.2 fish species, while MT and MC stations supported averages of 21.3 and 25.0 species, respectively.

Significantly more species of fish were collected at stations in the Vermilion basin than were collected at stations in the LaMoine basin. Stations in the Mackinaw basin supported significantly more species of fish than either the Vermilion or the LaMoine basin (Fig. 3; Table 2). Stations in the LaMoine basin supported an average of 15.4 fish species. An average of 20.9 species of fish were collected from the Vermilion basin, and stations in the Mackinaw supported an average of 24.6 species.

The species presence-absence data provided marginal support for the idea that the composition of fish communities at MT stations is more similar to MC stations than that of HT stations is to MC stations. Cluster analysis identified three relatively distinct groups of stations in the Vermilion River basin (Table 3). Group 1 consisted of five HT stations: an MT station, and station 84, categorized as an MC site but located a short distance downstream of a small urban area. Among these stations, the most numerically dominant species were the bluntnose minnow (*Pimephales notatus*), the sand shiner (*Notropis stramineus*), the spotfin shiner (*N. spilopterus*), and the creek chub (*Semotilus atromaculatus*). Ten stations made up Group

TABLE 4. Comparison of least-squares fits for geomorphic variables and transformed (natural log) fish species richness at sampling stations in the LaMoine, Mackinaw, and Vermilion basins. All coefficients were positive. Geomorphic variables compared included stream order, link magnitude, downstream link (D-link), and drainage area. SE is the standard error of the estimate.

Basin	Parameter	df	F	p	R ²	SE
LaMoine	Order	19	8.41	0.010	0.31	0.311
	Link	19	8.54	0.009	0.39	0.290
	D-link	19	11.29	0.004	0.39	0.290
Mackinaw	Order	17	3.64	0.075	0.17	0.334
	Link	17	5.18	0.037	0.32	0.303
	D-link	17	8.16	0.011	0.45	0.273
Vermilion	Order	22	6.83	0.016	0.25	0.298
	Link	22	12.25	0.002	0.37	0.273
	D-link	22	17.33	0.000	0.45	0.254
	Drainage area	22	11.439	0.003	0.35	0.276

TABLE 5. Average gradient (m/km) of the total length and the upper and lower halves of HT and MT tributary streams in each of three drainage basins.

Drainage basin	Total stream reach	Upper half of stream reach	Lower half of stream reach
Mackinaw			
MT tributaries	1.390	1.471	1.309
HT tributaries	1.716	2.499	1.415
LaMoine			
MT tributaries	1.549	1.998	1.131
HT tributaries	1.268	1.634	0.820
Vermilion			
MT tributaries	2.168	2.502	2.181
HT tributaries	2.044	4.669	0.888

2: five MT stations, three MC stations, and two HT stations (Table 3). The numerically dominant taxa among these stations were the longear sunfish (*Lepomis megalotis*), the bluntnose minnow, the striped shiner (*N. chrysocephalus*), and the johnny darter (*Etheostoma nigrum*). Group 3 was composed of four MC stations and one MT station. The dominant taxa at these stations were the spotfin shiner, the bluntnose minnow, the northern hog sucker (*Hypentelium nigricans*), and the central stoneroller (*Camptostoma anomalum*). The similar clustering of stations from MT and MC treatments into Groups 2 and 3 indicates that the composition of MT stations is generally more similar to MC stations than that of HT stations is to MC stations (Table 3).

Three groups of stations were identified in the Mackinaw River basin. The composition of MT stations was only marginally more similar to MC streams than was that of HT stations relative to MC stations (Table 3). All MC stations were categorized into Group 1 along with station DKB-01, an MT station. The dominant taxa at these stations were gizzard shad (*Dorosoma cepedianum*), golden redhorse (*Moxostoma erythrurum*), and emerald shiner (*N. atherinoides*). Group 2 was composed of both MT and HT stations, while Group 3 was composed entirely of HT stations (Table 3). Bluntnose minnows, central stonerollers, and striped shiners dominated most stations in Group 2, while central stonerollers, bigmouth shiners (*N. dorsalis*), bluntnose minnows, and striped shiners were numerically dominant at most of the Group 3 stations.

Similar to the Mackinaw basin, all MC stations in the LaMoine basin were categorized into a single group along with

TABLE 6. Results of three fully factorial, two-way ANOVAs on total stream gradient, the gradient in the upper half of a stream's length, and the gradient in the lower half of a stream's length with respect to tributary spatial location (Location) and drainage basin (Basin). Because no significant interactions were found, only the results on the main effects are reported.

	SSQ	df	MSQ	F	p
Total stream length					
Location	0.003	1	0.003	0.000	0.990
Basin	60.011	2	30.005	1.959	0.167
Error	306.286	20	15.314		
Upper half of stream					
Location	201.418	1	201.418	1.264	0.274
Basin	407.097	2	203.548	1.277	0.301
Error	3186.804	20	159.340		
Lower half of stream					
Location	45.340	1	45.340	3.080	0.095
Basin	36.854	2	18.427	1.252	0.308
Error	294.450	20	14.723		

TABLE 7. Results of one-way ANOVA on physical habitat parameters with respect to station treatments in the Mackinaw and Vermilion rivers. Habitat parameters analyzed from the Mackinaw included mean depth, mean velocity, and percent instream cover. Habitat parameters from the Vermilion included hydraulic diversity, mean substrate particle size, and the coefficient of variation in station depth.

Basin	Physical habitat parameter	F_{df}	p	Post hoc results
Mackinaw	Mean depth	$F_{2,12} = 0.654$	0.54	
	% instream cover	$F_{2,12} = 3.736$	0.06	
	Mean velocity	$F_{2,12} = 6.370$	0.02	HT < MC, MT = MC, HT = MT
Vermilion	Hydraulic diversity	$F_{2,14} = 1.247$	0.32	
	Mean substrate particle size	$F_{2,17} = 1.096$	0.36	
	CV depth	$F_{2,14} = 1.074$	0.37	

TABLE 8. Mean daily discharge (Q) and the associated coefficient of variation in daily discharge ($CV(Q)$) for the period April 20 to November 15, 1985, at stations DHT, 95, and DMC in the Vermilion basin.

Station	Location	Treatment	Mean Q (cfs)	$CV(Q)$
95	Jordan Creek	MT	8.9	196.44
DHT	Upper Salt Fork	HT	125.8	217.15
DMC	Vermilion River	MC	1210.8	218.20

station DGL-08, an HT station (Table 3). The dominant taxa at most stations in this group were the common carp (*Cyprinus carpio*), emerald shiner, gizzard shad, and the green sunfish (*L. cyanellus*). Group 2 was composed of only HT and MT stations that were dominated by green sunfish, red shiner (*N. lutrensis*), bluntnose minnow, and blackstripe topminnow (*Fundulus notatus*).

These results reflect the differences in the composition of the fish communities inhabiting stations in large rivers compared with stations located in tributaries. Consistently, the greatest similarity in composition was between HT and MT stations. In the Mackinaw and Vermilion basins, there was a tendency for the composition of some MT streams to be more similar to MC stations than was that of HT streams to MC stations.

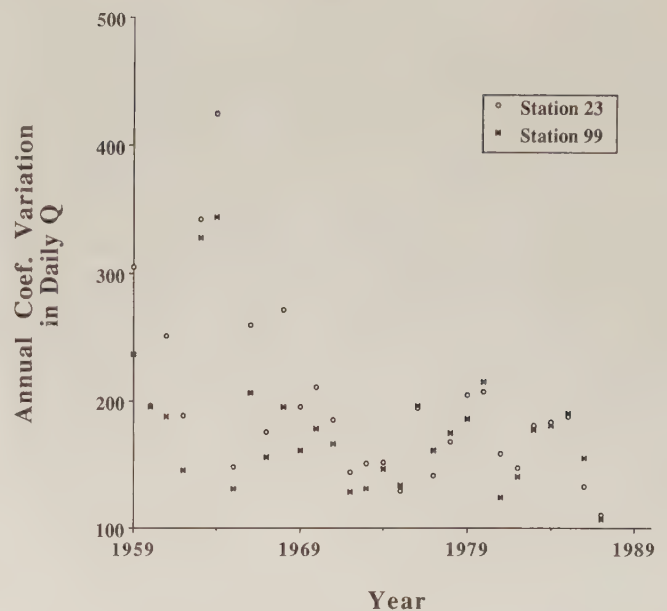


FIG. 4. Mean annual coefficient of variation in daily discharge at station 23, representative of HT stations, and station 99, an MC station, in the Vermilion basin over a 29-yr period (1959–88).

Within all three basins, a positive relationship was found between species richness and the geomorphologic parameters used to reflect stream size (Table 4). Stream order consistently provided the poorest relationship to fish species richness (Table 4). Without exception, the best relationship occurred between species richness and the downstream link which accounted for 39% of the variation in the LaMoine basin, 45% in the Mackinaw basin, and 45% in the Vermilion basin (Table 4).

Habitat Diversity

The gradient of tributary stream channels ranged from a minimum of 1.268 m/km to a maximum of 2.168 m/km (Table 5). Despite this range, no significant differences were found in the gradient of the total length of tributary streams attributable to either their location in a drainage network (HT versus MT) or to drainage basins (Table 6). Because the more headwater reaches of a stream may differ from the downstream reaches, we also examined the influence of drainage basin and stream network location on the gradients that occurred in the upper half of the tributaries and gradients that occurred in the lower half of the tributaries. We found no significant influence of either of these main effects on gradients in the upper or lower half of the tributaries (Table 6). Gradient in the upper half of the tributary streams ranged from 1.471 to 4.669 m/km and in the lower half of tributary streams ranged from 0.820 to 2.181 m/km (Table 5).

We analyzed habitat data for three parameters in the Vermilion and Mackinaw basins. Statistically significant differences associated with station treatments effects were found only in the average velocity at stations in the Mackinaw basin (Table 7). Further testing revealed that the average velocity at MC stations was significantly higher than velocities at HT stations (Table 7). No significant differences were found in average velocities between MT and HT stations; nor were significant differences found in other habitat parameters that corresponded to differences in fish species richness among sampling stations.

Discharge

Mean daily discharge increased with drainage area (Table 8). In 1985, average daily discharge at station 95 was $0.252 \text{ m}^3/\text{s}$, substantially lower than the mean of $0.445 \text{ m}^3/\text{s}$ recorded during the 1988–90 period, suggesting that average discharge in 1985 may have been lower than normal. Despite this lower discharge, there was no significant desiccation of stream reaches observed during our sampling. The $CV(Q)$ at station 95 was 194.2 during the subsequent 1988–90 period, very similar to that estimated in 1985 (Table 8).

The $CV(Q)$ for the period April–November 1985 was similar among stations regardless of network location and stream size. Comparison of the transformed annual $CV(Q)$ s over the 29 yr of record (Fig. 4), however, indicated that discharge at station DHT was significantly more variable than the mean daily discharge at station DMC ($p < 0.0001$, $df = 28$, paired t -value = -20.97). The time series of $CV(Q)$ was similar between stations (Fig. 4). Further, the difference in the $CV(Q)$ between the two stations was much less during 1985 than the average difference during the 29-yr period of record. This finding is consistent with the results reported for station 95 during the 1988–90 period.

Discussion

The tendency for species richness to increase with area has been widely observed by community ecologists (Schoener 1987) and represents a basic tenet of island biogeography theory (MacArthur and Wilson 1967). We found a significant and positive relationship between fish species richness and several geomorphological metrics that reflect stream size (Table 4). These findings are consistent with the results of numerous other studies on lotic fish communities (Angermeier and Schlosser 1989; Horwitz 1978; Larimore and Smith 1963; Sheldon 1968; Rahel and Hubert 1991). We also examined the relationship between fish species richness and the link magnitude of the next downstream confluence (D-link). The D-link metric accounted for the greatest proportion of variance in species richness among stations in each of the three drainage basins examined (Table 4). Further, it is the only one of the four geomorphologic metrics employed that incorporates downstream influences (e.g. proximity to primary channel) and relative spatial location within a drainage network in its computation (the higher the value, the more downstream is the confluence within the network). The magnitude of the other metrics was solely dependent upon upstream morphology and channel density. Thus, these results suggest that downstream, as well as upstream, factors can significantly influence the structure of stream fish communities. We do not necessarily advocate the use of the D-link metric, but rather, have developed and employed it here to reflect the importance of both downstream and upstream processes on the structure of stream fish communities.

Empirical evidence (e.g. Larimore and Smith 1963; Horwitz 1978; Angermeier and Schlosser 1989; Jackson and Harvey 1989) has demonstrated that substantial variability can occur in the fish species–area relationship within a system. Our analyses further indicate that headwater streams (HT streams) support fewer species of fish than do similarly sized main channel tributaries (MT) located more downstream in a drainage network (Table 2; Fig. 3). Thus, the location of tributary streams in a drainage network may explain a proportion of the unexplained variance in earlier stream studies.

We found no significant differences in species richness between MC and MT stations (Fig. 3) in either the LaMoine

or the Mackinaw basin despite large differences in the size of the two station treatments (e.g. greater width and depth per channel length). In the Vermilion basin, average species richness at MT stations was also more similar to that of MC stations than to that of HT stations (Fig. 3). These data suggest that stream size is not the sole determinant of the species richness of fish communities in warmwater streams. The similarity in species richness between MT and MC stations indicates that the location of a stream channel within a network may provide a general template of fish community structure in warmwater systems by regulating potential species richness. Such spatial patterns are not limited to streams. For instance, Jackson and Harvey (1989) have reported a significant association between geographical proximity and fish composition among lakes in the Great Lakes region of North America.

Schlosser (1982) presented evidence that fish community structure within a single stream, Jordan Creek, Illinois, was consistent with the concept that community organization was associated with spatial and temporal changes in channel morphology and resource availability. We also observed a gradient of increasing downstream species richness in Jordan Creek (stations 92, 95, and 96) (Fig. 2) similar to that reported by Schlosser (1982). We further found that the influence of network location on fish species richness was consistent across the drainage basins examined despite substantial interbasin differences in fish species richness (Table 2; Fig. 3). When viewed with Schlosser's work, these results suggest that gradual changes in such habitat characteristics as depth, velocity, and substrate size may modify fish community structure locally or within individual stream reaches; other larger scale factors such as network location may influence the number of species available for habitation.

As would be expected on the basis of the similarity in stream size and habitat conditions, the taxonomic compositions of fish communities at MT and HT stations were generally more similar to one another than they were to communities inhabiting MC streams. In several instances, however, the community composition of MT stations was similar to that at MC stations (Tables 3 and 4). The fact that the composition of fish communities of smaller MT stations can be more similar to those of large river stations than to those of similarly sized HT streams reflects a linkage between MT and MC systems. To date, the importance of such a linkage has generally been restricted to seasonal fish migrations associated with reproductive requirements of several species (e.g. Hynes 1970; Schlosser 1982; Winston et al. 1991) or has only been hypothesized (Gorman 1986). The significantly higher number of species at MT stations along with the potential for the community composition during nonreproductive periods to be similar to that at MC stations supports our contention of the importance of stream location on fish community structure.

It could be argued that the criteria used to distinguish between HT and MT streams were inappropriate or subjective. These criteria were arrived at and applied to the Mackinaw and LaMoine basins following the detection of a pattern in species distribution within the Vermilion basin. It was our intention to make them as simple and generally applicable as possible. Using these criteria led us to discern a similar species richness pattern among three drainage basins ranging in size from 2312 to 3522 km^2 , suggesting that the criteria may be generally appropriate for addressing similar questions in other low-gradient, midwestern watersheds. Undoubtedly, a more formal conceptual model of species richness in warmwater drainage networks is needed for the future.

Differences in species richness among similarly sized tributaries that can be attributed to location within a stream network have significant applied and basic implications. For instance, in warmwater systems, fish communities inhabiting headwater streams can be mistakenly classified if evaluation criteria are based upon information gathered from streams located lower in the drainage network, even though the streams are of similar size. Thus, from a resource management perspective, headwater tributaries should not be expected to have as speciose a fauna as similarly sized (stream order or drainage area) tributaries located lower down in the drainage network.

Using information contained within Matthews and Heins (1987), Schoener (1987) made one of the first attempts at incorporating temperate stream fish communities into a classification of "simila communities" with respect to organismic and environmental characteristics. Simila communities consist of a set of species in some place similar in their organismic characteristics (Schoener 1987). Compared with other communities, stream fish communities were found to be distinctly intermediate in most environmental characteristics. The exception appeared to be associated with spatial fragmentation; on a continuum of broken to continuous, stream fish communities were classified as distinctly more continuous than other communities. Our results suggest that the location of streams within a drainage network could influence the perception of the degree of fragmentation of fish communities inhabiting warmwater streams. For instance, comparison of data from MT stations with those from MC stations could lead one to conclude that warmwater stream communities are less fragmented than expected on a basinwide scale. Alternatively, a distinctly more fragmented view of streams could be obtained if one were to compare HT with MT or MC streams. These data further suggest that basin-level processes can significantly influence local community structure; this finding is consistent with Ricklefs' (1987) call for ecologists to broaden their concept of community processes.

Potential Mechanisms Responsible for Observed Pattern

Because hydrological conditions have been shown to be instrumental in structuring warmwater stream fish communities (e.g. Larimore et al. 1959; Horwitz 1978; Ross et al. 1985; Schlosser 1985), we viewed the variability of the Vermilion River hydrology data in the context of the comparable historical record of stream discharge. Over a 29-yr period, average annual discharge was significantly more variable at the HT station than at the MC station (Fig. 4). We were unable to find evidence of a difference in the variability in discharge between MT and HT streams. A greater variability in smaller streams is consistent with earlier results (e.g. Horwitz 1978) and reflects the generally more variable nature of environmental conditions in smaller stream systems (Grossman et al. 1982; Rahel and Hubert 1991). While possibly contributing to the differences in species richness between HT and MC streams, variation in discharge does not by itself account for the differences in species richness between HT and MT streams.

Angermeier and Schlosser (1989) suggested three potential mechanisms to explain the documented species-area relationship among stream fishes: (1) increased diversity in habitat type is associated with increased area (Williams 1964); (2) larger areas support more individuals; thus, the pool of available species is sampled more completely (Connor and McCoy 1979); and (3) differential rates of immigration and extinction have been found to exist (MacArthur and Wilson 1967). The ability of each of these mechanisms to account for the observed spe-

cies-area relationship among fish continues to be debated (Schoener 1987; Power et al. 1988). Although our study was not designed to conclusively test the relevance of each of these mechanisms, available information does permit us to speculate on their applicability to account for the watershed patterns in species richness.

HT stations had significantly fewer species than either MT or MC stations. The Connor and McCoy hypothesis would predict that HT streams would have significantly fewer individuals than either MC or MT stations. This hypothesis would also predict no differences in the density of fish between MT and MC stations or significantly fewer numbers of individuals at MT stations than at MC stations. As previously reported, we found no significant effect of the number of individuals when included as a covariate in the ANCOVA (Table 2). While sample size is unquestionably important, especially when concerned with rare species (Sheldon 1987), the evidence available to us does not support the Connor and McCoy hypothesis as a potential mechanism.

Sampling stations in the Vermilion basin were selected to be as similar to one another as possible, including retaining the same proportion of channelized to unchannelized stations in each of the HT and MT treatments and incorporating a similar distribution of stream sizes that could influence habitat conditions (Lanka et al. 1987). Comparison of the variability in stream discharges within the Vermilion basin (Table 8) also indicated minimal differences between MT and HT streams during the 1985 study period.

To further address the possibility that differences in habitat conditions between MT and HT streams may have accounted for the observed differences in species richness of fish, we compiled information on stream gradient and analyzed it along with published data on inchannel physical habitat. We were unable to find evidence of a difference in either gradient or inchannel physical habitat between HT and MT streams. We recognize that our physical habitat data were restricted in both extent and parameters measured. For instance, pool habitats have been shown to influence the species richness of warmwater stream fish communities (Sheldon 1968; Schlosser 1982, 1987). Although we found no differences in parameters associated with the number of pools (i.e. mean depth and gradient), it is conceivable that a greater number of pools per unit stream length may have occurred in MT streams compared with HT streams. Future basin level studies should address this question in greater detail. Because our habitat data were not exhaustive in scope, we do not reject the possibility of there being significant differences in the habitat conditions between HT and MT streams. We were, however, unable to detect evidence to support such an hypothesis.

The immigration-extinction hypothesis (MacArthur and Wilson 1967) appears to be the most plausible mechanism for the observed differences in species richness between HT and MT stations. It has been established that physical conditions in smaller streams are more variable than those in larger systems. For instance, smaller streams are vulnerable to periodic droughts that have been shown to impact stream fish communities (Larimore et al. 1959; Ross et al. 1985; Osborne et al. 1991). Additionally, discharge is more variable in tributary streams than in larger main channel reaches (Fig. 4). Horwitz (1978) has identified gradients of fish species richness that were correlated with patterns of flow variability, thereby providing a linkage between discharge variability and the structure of fish communities. Further, larger stream channels maintain sufficient flow to support a diverse fish community even during

periods of severe drought (Osborne et al. 1991). Thus, larger streams could serve as refugia for fish that normally inhabit adjacent MT tributaries before flow becomes intermittent. Possibly more important (Sheldon 1987), larger streams may provide a pool of immigrants for subsequent recolonization following a disturbance.

An additional tenet of island biogeography theory (Simberloff 1974; MacArthur and Wilson 1967) is that species richness should be greater on islands located closer to the colonizing source than on more distant islands of similar size. By definition, MT streams flow into larger MC systems whereas HT sites are more remotely located from the potential refuge of the main channel. The significantly greater number of species at MT stations compared with the number at HT stations is consistent with the concept that the immigration-extinction hypothesis is an important mechanism accounting for the differences in species richness between HT and MT streams. The significant positive relationship between species richness and D-link also supports such a premise. Sheldon (1987) has provided evidence in support of immigration as an important determinant of fish community structure in warmwater streams. The similarity of the composition of the fish communities at some MT stations to MC stations in the Vermilion and Mackinaw basins (Table 3) further supports this contention. Thus, these data support the general concept that at the watershed scale, fish species richness in warmwater tributary streams is influenced by the frequency of disturbance and by emigration-colonization dynamics.

The fact that we suggest that frequency of disturbance and colonization dynamics are important mechanisms dictating fish community structure at the watershed level does not minimize the potential importance of biological interactions that can significantly influence community structure at the local level or ephemerally (e.g. Schlosser 1985, 1987). Nor is it inconsistent with the importance of channel geometry and a greater frequency of pool habitats. Rather, these results suggest that large-scale features such as climate, geology, and spatial location within a drainage network provide the general template of environmental conditions within which biological interactions operate. These results are consistent with the concept of changes in community structure along environmental gradients (e.g. Schlosser 1987; Connell 1978; Karr and Freemark 1985) if one considers that gradients do occur within a larger spatial framework or time period. Recognizing that controlling mechanisms can vary within a spatial framework may also help explain the controversy over physical and biological regulation of stream fish community structure (e.g. Grossman et al. 1982; Rahel et al. 1984; Yant et al. 1984; Power et al. 1988). Undoubtedly, both factors can and do modify community structure. The important ecological questions become within what temporal and spatial framework do physical and biological interactions manifest themselves and to what degree? Addressing such questions will obviously require more detailed multiscale experiments and approaches.

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Large Woody Debris and Salmonid Habitat in a Small Coastal British Columbia Stream

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Sections of a small coastal British Columbia stream that had previously been cleaned of large woody debris (LWD) were compared with sections where most debris was left and with others where debris had been relatively undisturbed for at least 40 yr. Three sections where debris had been removed had simple habitat that was less sinuous, wider, and shallower and had less pool volume and overhead cover than four sections with more complex habitat where debris was retained. Habitat in four relatively undisturbed sections was generally similar to complex sections. Most pools in all sections were scour or plunge pools formed by LWD or large roots oriented perpendicular to the flow or angled downstream. Standing crop (kilograms per hectare) and individual weights of age 1+ and older coho salmon (*Oncorhynchus kisutch*) and cutthroat trout (*O. clarki*) were significantly greater ($P < 0.02$) in complex than in simple sections. Biomass of age 1+ and older salmonids was closely related to section pool volume ($r^2 = 0.92$, $P = 0.0006$). Projections based on this model and average habitat conditions suggest that during 1990 a total of 8.0 kg of salmonid biomass, 5 times the current standing crop, was forgone in the 332-m simple reach due to prior debris removal.

Des segments d'un petit cours d'eau côtier de la Colombie-Britannique dans lesquels on a récemment retiré les gros débris de bois ont été comparés à des segments où la plupart des débris ont été laissés en place, et à d'autres dont les débris n'ont pour ainsi dire pas été touchés depuis au moins 40 ans. Par rapport à quatre segments où les débris n'ont pas été retirés et présentant un habitat complexe, trois segments dont les débris n'ont pas été retirés et présentant un habitat complexe, trois segments dont les débris avaient été retirés présentaient un habitat simple, moins sinueux, plus large, moins profond et ayant un volume de fosses et un couvert végétal plus faible. L'habitat dans quatre segments relativement peu perturbés était généralement semblable à celui des segments à habitat complexe. La plupart des fosses dans tous les segments étaient des fosses creusées par affouillement ou par une chute d'eau attribuables à la présence de gros débris de bois ou de grosses racines perpendiculaires au sens d'écoulement ou formant un angle ouvert vers l'aval. La biomasse totale (kilogrammes par hectare) et les poids individuels des saumons cohos (*Oncorhynchus kisutch*) et des truites fardées côtières (*O. clarki*) d'âge 1+ et plus dans les segments complexes étaient supérieurs ($P < 0,02$) à ceux mesurés dans les segments à habitat plus simple. La biomasse des salmonidés d'âge 1+ et plus était fortement corrélée au volume total des fosses des segments ($r^2 = 0,92$, $P = 0,0006$). Les prévisions fondées sur ce modèle et sur les conditions moyennes des habitats laissent entendre qu'au cours de 1990, la biomasse de salmonidés dans le segment simple de 332 m a été réduite de 8,0 kg, ce qui représente 5 fois la biomasse totale actuelle, après qu'on y ait retiré des débris.

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Large woody debris (LWD) that enters forested streams plays important roles in shaping channel morphology (Keller and Swanson 1979; Bisson et al. 1987; Robison and Beschta 1990a, 1990b). Channels shaped and maintained by LWD in turn form the habitat template (Southwood 1977) to which salmonids and other aquatic biota in Pacific Northwest streams have evolved (Sullivan et al. 1987).

Human activity in the riparian forest frequently reduces the amount of LWD that enters or persists in stream channels. This occurs either indirectly by deforesting the riparian zone that is the source of LWD, or by direct removal of debris from streams. Both are especially detrimental in streams near urban areas where standing dead trees are removed due to the perceived hazard to human life and property, and fallen debris is more or less continuously removed for firewood or "cleaned up" for

misguided aesthetic reasons. This cumulative debris removal is expected to greatly alter channel morphology and the biotic processes that depend on it.

We studied the role of LWD in forming salmonid habitat in a stream that traverses second-growth forest near the city of Vancouver, British Columbia. Our primary hypothesis was that sections where debris had been removed would have fewer pools and harbor fewer salmonids compared with those left uncleaned.

Study Area

The Musqueam–Cutthroat Creek system (Fig. 1), adjacent to the city of Vancouver, British Columbia, drains a 730-ha watershed that is underlain by glacial till and sediments of marine origin (Armstrong 1956; Anonymous 1981). Both

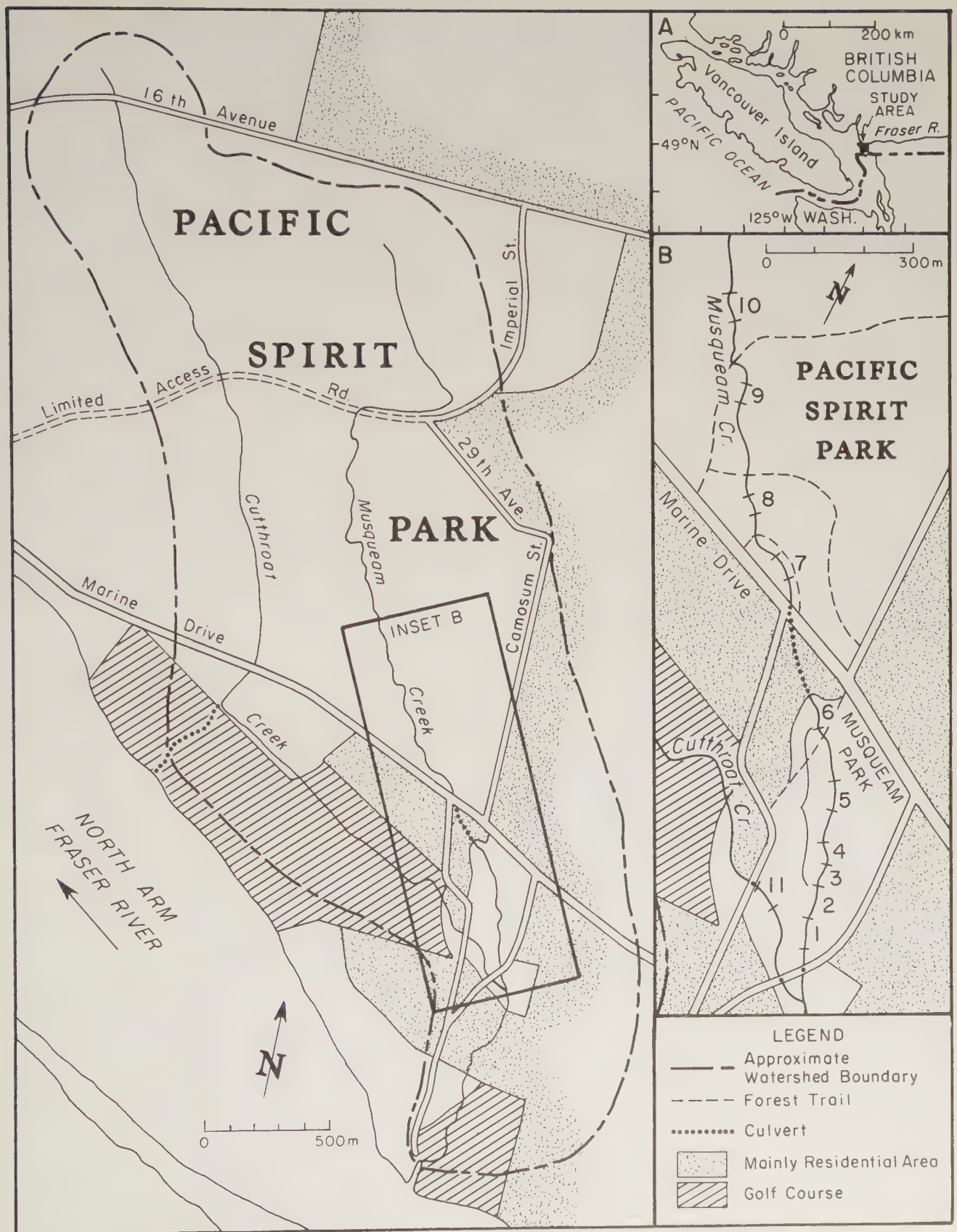


FIG. 1. The Musqueam-Cutthroat Creek watershed. Stippling indicates lands that are mainly residential, and hatching shows golf courses. Inset A shows the location of the study area near Vancouver, British Columbia, and inset B shows the study sections in Pacific Spirit Park and Musqueam Park. The bracket in Musqueam Park shows the reach where LWD was removed. Long culverts beneath Marine Drive are probably impassable to all fish, and the culvert immediately upstream from section 11 is probably impassable to all but adult coho salmon (see text).

streams arise in bogs on the uplands (130 m above sea level) and originally downcut through 30-m-high coastal cliffs to a tidal marsh. Discharge varies from maxima in fall or winter that reach $6.5 \text{ m}^3 \cdot \text{s}^{-1}$ (Anonymous 1981) to late summer minima as low as $0.0002 \text{ m}^3 \cdot \text{s}^{-1}$ (Fausch 1990; Heggenes et al. 1991). Water temperatures range from about 1 to 20°C , with lower summer maxima in upstream reaches.

The streams are some of the last in the greater Vancouver area that support salmon or trout (Harris and Proctor 1989). Populations in the reaches studied are dominated by coastal cutthroat trout (*Oncorhynchus clarki clarki*) and juvenile coho salmon (*O. kisutch*). Western brook lamprey ammocoetes (*Lampetra richardsoni*) were abundant in section 1 (Fig. 1B), and a few prickly sculpin (*Cottus asper*) were also captured there.

Original vegetation was dominated by old-growth Douglas-fir (*Pseudotsuga menziesii*), western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and red alder (*Alnus rubra*). The region was selectively logged periodically from 1886 to 1950 (Norris 1973). No large slash fires occurred and little fire history is known. After 1912 the uplands became part of the University of British Columbia Endowment Lands forest, formally established in 1925. Recently, much of this area has been redesignated as Pacific Spirit Park. The land surrounding the high-gradient reach of Musqueam Creek downstream became Musqueam Park in the 1920s.

Urbanization near the downstream reaches of Musqueam and Cutthroat creeks has increased since the 1960s. Long steep culverts (279 m, 2.6–4.3% slope on Musqueam Creek) constructed beneath Marine Drive during the 1970s probably are impassable to fish, and another on Cutthroat Creek downstream from Marine Drive probably limits upstream passage of all but adult coho salmon (Fausch 1990). Only very small populations of resident cutthroat trout persist upstream from Marine Drive (Northcote and Hartman 1988; T. G. Northcote and K. D. Fausch, pers. obs.).

Cutthroat Creek was diverted into a tributary of lower Musqueam Creek during golf course construction several decades ago. Floods and base flows throughout the watershed have been made more extreme by lawn irrigation and tile and storm drainage associated with residential development. During spring and summer 1990, dissolved ions, heavy metals, and nutrients were generally 1.3–15 times higher downstream from Marine Drive than upstream due to drainage from roads and suburban lands, but no dissolved concentrations were high enough to pose known hazards to salmonids (Fausch 1990).

The second-growth forest upstream from Marine Drive has been relatively undisturbed since at least about 1950. In contrast, although 25–50 m of riparian forest remains along either side of the 541 m of stream that traverses Musqueam Park, most LWD was removed from the middle 332 m during park maintenance in the 1960s and 1970s (T. G. Northcote, pers. obs.). Leaning or fallen trees throughout the park are often removed to reduce hazards to park visitors.

Methods

We chose eleven 45- to 70-m stream sections (Fig. 1) to contrast LWD and fish habitat in the relatively undisturbed Pacific Spirit Park forest (PSP) with that in Musqueam Park sections downstream that had been either cleaned of LWD (simple) or not cleaned (complex). We also compared fish populations in the simple and complex sections, all of which held both cutthroat trout and juvenile coho salmon. Fish were not sampled in PSP sections because few occurred there.

Four PSP sections, located in the 1200 m of Musqueam Creek upstream from Marine Drive, were randomly chosen within four relatively homogeneous reaches delineated by forest trails. Each section began at a randomly selected distance from an adjacent trail, except in the most downstream reach (section 7) where only one 50-m length had relatively undisturbed wood debris. In Musqueam Park, three simple sections (3, 4, and 5) were located in the 332-m midreach that had been cleaned of most debris, and three relatively complex sections were located upstream (6) and downstream (1 and 2) of these (Fig. 1). A fourth complex section (11) was in lower Cutthroat Creek near its confluence with Musqueam Creek. All sections had similar flows ($0.0015 \text{ m}^3 \cdot \text{s}^{-1}$) when habitat and LWD were mapped and measured during 8–22 June 1990 (Fausch 1990). Angling was prohibited in Musqueam Park, and no evidence of angling was found in any of the reaches studied.

We analyzed differences in habitat, LWD, and fish populations among simple, complex, and PSP sections using a random-effects one-way analysis of variance (ANOVA), which is based on the assumption that the three types of sections were a random sample of more such types (Sokal and Rohlf 1981). Although not strictly true, we selected this analysis because inferences based on random-effects ANOVA are more conservative than for fixed effects, where the investigator purposely manipulates variables (e.g. LWD) to set up treatments. Furthermore, after completing the ANOVA, we calculated multiple comparisons among pairs of means using Bonferroni *t*-tests, while controlling experimentwise error rate at $P = 0.05$, which is also a conservative procedure.

Salmonid Habitat

In each stream section, wetted and bankfull widths were measured at transects perpendicular to flow, spaced at 1-m intervals in pools and at 2- or 3-m intervals in runs and riffles. The 3-m interval was used in the simple sections because most habitat consisted of long runs. Depth was measured at 0.5-m intervals along transects, beginning 0.25 m from the right stream bank facing downstream. The first transect was at half the transect interval from the downstream end of each habitat type, so depth measurements were assumed to represent conditions in cells 0.5 m wide and 1–3 m long.

Total length of each habitat unit (riffle, run, pool) was measured along the stream centerline. Pools were classified as scour, plunge, dammed, or backwater pools after Bisson et al. (1982) and the maximum depth measured. Length of undercut bank and overhanging log and rock cover that could conceal a 150-mm-long salmonid from overhead view was measured for each section. Structures that were at least 15 cm wide (10 cm for undercut banks, due to blind end), had a least 15 cm of water beneath them, and were no more than 15 cm above the water surface were included. In early August 1990 when surface flow had ceased in the PSP reach of Musqueam Creek, all pools in the most downstream 770 m were classified as before and their volumes and maximum depths measured.

The six sections in Musqueam Park were mapped with compass and tape using the deflection-angle-traverse method (Orth 1983). Section sinuosity was calculated from the maps as the distance along the centerline divided by the distance along a straight line between the section ends. Section gradients were determined by surveying with a level and leveling rod.

Large Woody Debris and Riparian Stands

The dimensions and orientation of all pieces of LWD (≥ 1 m long, ≥ 10 cm mean diameter) that were at least partially within

or above the bankfull channel were measured for each section, and in the reaches between sections in Musqueam Park. Habitat in the 19 m downstream from section 1 had been modified and was not included. The total length of each LWD piece, the length within or above the bankfull channel (hereafter termed within bankfull channel), and the diameters at each end and at the channel margins were measured using a tape and tree calipers. Pieces of debris that formed pools were related to habitat measurements.

Volumes were calculated for individual pieces of debris ≥ 2 m long by assuming cylindrical shape and using length and average diameter (Lienkaemper and Swanson 1987). The volume of debris within the bankfull channel and the total debris volume were determined for each section. Biomass within the bankfull channel was estimated from an average conifer wood density of $0.40 \text{ Mg} \cdot \text{m}^{-3}$ (Harmon et al. 1986) because relatively little debris in channels was hardwood. Mean diameters and lengths of debris were calculated for each section using geometric means because of skewed distributions (Bilby and Ward 1989). Mean volume was calculated as a cylinder with geometric mean diameter and length.

In addition to total and bankfull LWD volume and biomass, we also calculated the number and within-channel volume of pieces that directly influenced channel morphology. We used our maps and field notes for the sections in Musqueam Park to identify only those pieces that deflected flow and caused scour or fill at flows of bankfull or less, or supported stream banks so that pools could form. We thus eliminated pieces suspended above the channel at bankfull flow or lying loose within the channel (often parallel to flow and along the bank) and not causing scour at any flow.

The diameter at breast height (DBH at 137 cm above the ground) and distance from the stream bank of each tree and stump within 30 m of the channel (Murphy and Koski 1989) throughout the 541-m Musqueam Park reach were measured to estimate future (and past) riparian sources of LWD. Each tree and stump ≥ 20 cm DBH was identified to species and measured with a steel diameter tape.

Fish Populations

Fish populations in the seven simple and complex sections downstream from Marine Drive were measured between 5 and 12 April 1990 before most age 1+ and older coho salmon had smolted. Flow was low and water temperatures ranged from 8 to 11°C , so electrofishing was effective and fish mortality low. Salmonid abundance was estimated by three- or four-pass removal electrofishing with a Smith-Root Type VIII A backpack electrofishing unit operated at 250–850 V. Voltage was increased in deep pools. The unit delivered square-wave pulsed DC via a 15-cm-diameter wand-mounted ring anode and a 20×30 cm metal screen cathode that trailed behind the operator, who also carried a net.

Section ends were blocked with fine-meshed seines (≤ 6.4 mm), and the bottom was lined with gravel or cobble to ensure population closure. The downstream block net of section 6 was removed by adolescents before the second pass but was reinstalled within 20 min, after which work continued. All habitat in each section was carefully electrofished with the same effort on each pass, an important assumption of removal estimators (Riley and Fausch 1992). Work was scheduled so that 1–2.5 h elapsed between passes.

Fish from each pass were retained in separate buckets and later anesthetized (2-phenoxyethanol), measured (total length, TL), and weighed (nearest 0.1 g, Ohaus model C300 electronic

balance). Weights of age 1+ and older salmonids from two sections were estimated from species-specific length–weight regressions based on all weighed fish because of a balance malfunction. The weight of one other cutthroat trout that exceeded the 300-g balance capacity was similarly estimated.

Population estimates were calculated using the maximum-likelihood generalized removal estimator of Otis et al. (1978) via the computer program CAPTURE (White et al. 1982). Log-based confidence intervals (Burnham et al. 1987, p. 212) were calculated to ameliorate the slight negative bias and low coverage normally associated with intervals for this estimator (Riley and Fausch 1992). Separate population estimates were calculated for age 1+ and older coho presmolts (>60 mm TL) and cutthroat trout (>70 mm). Section biomass was calculated as the product of the population estimate and mean weight for each species. Confidence intervals were determined using a finite population correction based on numbers of fish weighed. Recently emerged age 0 coho averaged only 36–38 mm TL during this period ($n = 24$ –50 per section for five sections that had age 0 fish). Their abundance was not estimated due to low capture efficiency and to prevent high mortality.

Results

Salmonid Habitat

The simple and complex sections in Musqueam Park differed markedly in sinuosity, pool volume, bankfull width, depth, and overhead cover for fish provided by undercut banks and overhanging logs (Table 1). The three sections with simple habitat had lower sinuosity, were wider and shallower, and had little cover for fish when compared with the four complex sections. Pools made up 9–25% of the total volume at base flow in the simple sections but were 61–69% of volume in the complex sections. Differences in percent pool volume (arcsine square-root transformed), width, depth, and cover were significant ($P < 0.05$ experimentwise error rate) when tested using Bonferroni *t*-tests for unequal variance, after one-way random-effects ANOVA.

The four randomly chosen PSP sections upstream of Marine Drive also had deep and relatively complex habitat. Pools comprised much of the total volume (44–58%), channels were narrower and deeper than in most downstream sections, and overhead cover was plentiful. The decrease in width, increase in depth (compared with simple sections only), and change in pool volume were significant, but the increase in overhead cover was not because of high variation. Much of the variation was due to section 7 which was wider and shallower than the other PSP sections, probably due to water and sediment contributed by an intermittent tributary entering immediately upstream. Although decreased width in sections 8–10 upstream may have been partially due to lower bankfull flows, increased depth was probably due to more stable banks and more LWD (see below).

Large Woody Debris

Total abundance and volume of LWD and estimated biomass within the bankfull channel were variable and similar for the simple and complex reaches in Musqueam Park, but generally greater in PSP sections (Table 2). For example, sections in the simple and complex reaches had 3–30 pieces per 100 m of stream whereas the upstream PSP sections had 26–60 pieces per 100 m. None of the differences in total debris abundance, volume, or biomass was significant by ANOVA due to high variance, although the difference in abundance between the PSP and complex sections was nearly so ($P = 0.0525$).

TABLE 1. Characteristics of physical habitat in simple, complex, and the relatively undisturbed PSP sections of Musqueam and Cutthroat creeks, June 1990. Weighted means and standard errors (SE, n = total sample size) were calculated for bankfull widths and depths, based on the proportion of section length (for width) and area (depth) made up by each habitat type. Significant differences ($P \leq 0.05$ experimentwise error rate, Bonferroni t -tests) among reaches for means of four habitat variables are denoted by different letters. Means followed by the same letter are not significantly different.

Section	Sinuosity	Gradient (%)	Length (m)	Volume (m ³)	% pool		Mean bankfull width (m) (SE, <i>n</i>)	Mean depth (cm) (SE, <i>n</i>)	Overhead cover (m ² ·100 m ⁻¹)
					Area	Volume			
Simple: Musqueam Park									
3	1.03	1.9	53	9.1	7	19	5.3 (0.38, 18)	6.2 (0.42, 103)	5.7
4	1.03	1.5	45	6.1	11	25	5.1 (0.50, 19)	6.4 (0.33, 90)	2.0
5	1.07	4.1	53	6.2	4	9	5.8 (0.29, 19)	4.8 (0.27, 95)	1.7
Mean						17.7 a	5.4 a	5.8 a	3.1 a
Complex: Musqueam Park									
1	1.24	2.1	70	14.6	43	64	3.4 (0.16, 45)	10.2 (0.51, 177)	22.7
2	1.29	1.5	69	13.0	35	61	3.6 (0.17, 45)	10.3 (0.48, 165)	14.3
6	1.37	4.0	51	9.0	42	62	3.1 (0.18, 34)	9.2 (0.48, 145)	23.9
11	— ^a	3.1	53	13.7	45	69	3.5 (0.24, 32)	12.5 (0.69, 159)	17.2
Mean						64.0 b	3.4 b	10.6 b	19.5 b
Undisturbed: Pacific Spirit Park									
7	—	1.2	48	8.3	27	46	3.0 (0.17, 29)	11.5 (0.63, 88)	11.3
8	—	0.6	46	11.7	27	44	2.4 (0.08, 27)	16.4 (0.95, 91)	20.2
9	—	0.7	48	12.7	43	51	1.9 (0.07, 31)	16.8 (0.87, 94)	37.1
10	—	0.5	48	13.4	44	58	2.2 (0.16, 33)	20.0 (0.87, 100)	36.7
Mean						49.7 c	2.4 c	16.2 b	26.3 ab

^aIndicates that no map was drawn, so sinuosity could not be estimated.

The number of pieces of LWD that directly influenced channel morphology was greater in complex sections (except section 2) than in simple ones. However, the total volume of these pieces that was within the bankfull channel was variable within reaches, and neither difference was significant due to high variability ($P > 0.05$ by t -test for unequal variance). In sections 2, 3, 4, 5, 5–6, and 6, $\geq 63\%$ of this volume was contributed by one large piece of LWD. Geometric mean LWD diameters, lengths, and calculated mean volumes were also variable and similar among the three reaches, except that the most upstream PSP section had primarily long pieces of debris of moderate diameter.

The distribution of LWD lengths in all sections was skewed toward short pieces (Fig. 2), although PSP sections had a higher percentage of longer pieces than those downstream. Overall, 36% of 104 LWD pieces in PSP sections were ≥ 8 m whereas only 19% of 85 pieces in the simple reach and 2% of 57 pieces in the complex reach of Musqueam Park were this long.

How important was LWD in forming pools? The majority of both number and volume of pools was formed either by LWD or large root masses of standing trees or stumps. In all, 72% of pool volume in the seven simple and complex sections downstream from Marine Drive (reaches combined to increase sample size) and 80% of pool volume in PSP sections were formed by these two agents (Table 3). In the PSP sections, most volume was contributed by lateral scour pools around LWD or large roots (76% of total) whereas in the downstream sections, about 40% of the pool volume formed by debris was from plunge pools, perhaps due to generally higher gradient. Neither dammed nor backwater pools contributed substantial volume in either reach. Lateral scour pools around boulders or beneath undercut banks supported by roots of shrubs or grasses were

also important in both reaches, making up about 20% of the volume in each. Percentages of pools by number were similar to that by volume.

Maximum depths of pools were greater in PSP sections, even though the channel cross-sectional area was smaller and gradient lower there, probably due to more LWD and more stable banks. Pools in PSP sections averaged >40 cm deep whereas only plunge pools formed by LWD in the downstream reach were this deep on average, even though habitat in both reaches was measured at the same base flow.

By early August when surface flow had ceased upstream of Marine Drive, maximum depths had declined an average of 19 cm (SE = 4.9) in 9 of 12 pools in sections 7–10 that were measured in mid-June (the other three were dry). For the 20 pools that remained in the entire lowermost 770 m of the reach at this low flow, 96% of the volume was in pools formed by LWD or large roots, of which 81% consisted of lateral scour pools and 15% plunge pools. Maximum depths ranged 30–52 cm in 11 of the 18 pools formed by LWD, even though flow had ceased.

Most of the debris that formed pools downstream from Marine Drive was oriented perpendicular to the channel (59% was 90°; Fig. 3). Most of the debris forming pools in the PSP sections was oriented either perpendicular or angled downstream (86% was 90–135°).

Riparian Sources of Large Woody Debris

The stand of trees within 30 m either side of Musqueam Creek in Musqueam Park was dominated by relatively young, small trees (Fig. 4). Most were <70 cm DBH, and only 5 of the 583 trees ≥ 20 cm DBH were >100 cm in diameter. In

TABLE 2. Characteristics of LWD (≥ 10 cm in diameter and ≥ 2 m long) in simple and complex reaches, and the relatively undisturbed PSP sections of Musqueam and Cutthroat creeks, June 1990. Number of pieces and volume of debris that directly influenced channel morphology are referred to as "channel." Geometric means of length, diameter, and mean volume per piece were calculated due to skewed distributions (see text). Volumes were summed for individual pieces of debris, based on lengths within the bankfull channel and on total length. Biomass within the bankfull channel was based on average conifer density of $0.4 \text{ Mg} \cdot \text{m}^{-3}$ (Harmon et al. 1986). Significant differences ($P < 0.05$ experimentwise error rate, Bonferroni t -tests) among reaches for means of five debris characteristics are denoted by letters. Means followed by the same letter are not significantly different.

Section	Pieces·100 m ⁻¹		Geometric mean			Volume (m ³ ·100 m ⁻¹)		Bankfull biomass (Mg·ha ⁻¹)
	All	Channel	Diameter (m)	Length (m)	Volume (m ³)	Channel	Total	
Simple: Musqueam Park								
3	11	4	0.37	6.0	0.65	4.6 ^a	19.9	67
4	16	4	0.30	6.9	0.48	9.0 ^a	15.3	83
4-5	30	0 ^b	0.20	5.8	0.18	0.0	13.4	36
5	11	2	0.24	3.5	0.15	1.3	3.2	19
5-6	15	5	0.23	4.9	0.21	2.8	16.0	— ^c
Mean	16.6 a	3.0 a				3.5 a	13.6 a	51.2 a
Complex: Musqueam Park								
1	20	17	0.41	3.0	0.39	10.7	11.2	133
2	3	1	0.25	3.9	0.20	1.2	1.5	15
6	16	16	0.43	3.7	0.55	15.2	45.0	190
11	13	9	0.32	3.9	0.30	3.2	6.7	42
Mean	13.0 a	10.7 a				7.6 a	16.1 a	95.0 a
Undisturbed: Pacific Spirit Park								
7	60	— ^d	0.19	6.3	0.18	— ^d	25.4	142
8	26	—	0.29	3.7	0.24	—	16.6	74
9	40	—	0.32	5.9	0.48	—	45.9	448
10	42	—	0.27	12.6	0.69	—	85.0	274
Mean	42.0 a ^e						43.2 a	234.5 a

^aA large percentage (65% in section 3, 80% in section 4) of "channel" volume was contributed by one large piece of LWD that had little effect on flow.

^bAn additional eight pieces (12 per 100 m) of new, broken red alder LWD were wetted and eight more were suspended above the channel. All affected channel morphology little, and none was stable.

^cBiomass could not be estimated because section area was not measured.

^dNeither the number nor volume of pieces that influenced channel morphology could be calculated for PSP sections (see text).

^eDifferences between the number of LWD pieces in simple and PSP sections ($P = 0.08$) and complex and PSP sections ($P = 0.0525$) were significant at $P < 0.10$.

contrast, 17 fir and 11 cedar stumps of 100–240 cm DBH remained from the original stand, 6 of which were > 200 cm.

Fir and hemlock were relatively more abundant and hardwoods (primarily red alder) less abundant adjacent to the complex sections than to the simple ones. This may be partly due to steeper, drier slopes near several complex sections and partly due to a narrower riparian strip that supported primarily alder on one side of the simple midreach of Musqueam Park.

If half of the conifers > 20 cm DBH now growing within 30 m of the stream eventually fell across or into the channel and remained there for 100–200 yr, as has been estimated elsewhere in the Pacific Northwest (Murphy and Koski 1989), debris loads would be 37 pieces per 100 m of stream in the complex reach and 21 pieces per 100 m in the simple reach (based on total trees divided by reach lengths). These estimated conifer loads are probably conservative, given slope and other factors that bias fall direction toward the stream (Van Sickle and Gregory 1990), yet are relatively high compared with those now present in most of Musqueam Park (Table 2; 80% of pieces in section 4–5 were alder). The debris load estimate for the

complex reach is similar to the average for PSP sections where debris has presumably been disturbed little during the last 40 yr.

Salmonid Populations

Mosts age 1+ and older salmonids that could be captured by electrofishing were removed from each section in three or four removal passes (Table 4). The generalized removal estimator indicated that 58–95% ($\bar{x} = 78\%$) of the fish remaining were captured on each pass. As a result, standard errors of estimates were low, and the lower 95% confidence limit, total number of fish captured, and population estimate were equal for all cases. For 11 of 14 estimates the upper confidence limit was only zero or one fish more than the estimate.

Estimates and upper confidence limits probably slightly underestimate true population size either because capture probabilities decline for fish remaining (Bohlin and Cowx 1990) or because some fish cannot be sampled by electrofishing. Simulations indicate that the former bias is probably 5% or less for the range of capture probabilities reported here (Riley and Fausch 1992). Biomass estimates are precise because the entire

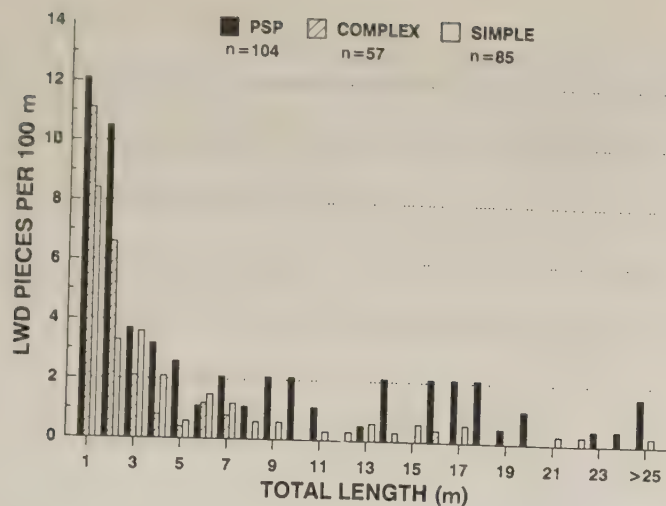


FIG. 2. Frequency of LWD pieces per 100 m of stream by 1-m length classes in PSP, complex, and simple sections of Musqueam and Cutthroat creeks. The total number of pieces measured in each reach is shown.

TABLE 3. Characteristics of pools of four types (after Bisson et al. 1982) created by LWD and other agents in reaches downstream and upstream of Marine Drive. Data for the seven simple and complex sections in the downstream reach were combined to increase sample size. Percentages of total pool volume (% V) and total numbers (% N) for each reach (n = sample size) and mean (SE) and range of maximum pool depths are shown.

Pool type	Downstream		Upstream	
	LWD or roots	Boulders, POM, ^a or other	LWD or roots	Boulders, POM, ^a or other
Scour				
% V	38	19	76	20
% N	39	25	67	25
Max. depth	28.1 (3.28)	27.5 (1.47)	51.3 (5.56)	45.7 (3.18)
Range	19–76	18–33	30–78	42–52
Plunge				
% V	29	8	4	0
% N	18	7	8	0
Max. depth	41.1 (5.24)	34.0 (7.23)	44 (—)	—
Range	21–64	22–47	44	—
Dammed				
% V	1	0	0	0
% N	2	0	0	0
Max. depth	23	—	—	—
Range	23	—	—	—
Backwater				
% V	4	0	0	0
% N	9	0	0	0
Max. depth	14.8 (5.88)	—	—	—
Range	1–28	—	—	—
Total				
% V	72	28	80	20
% N	68	32	75	25
n	30	14	9	3
Volume (m ³)	26.0	10.0	18.2	4.4

^aFine particulate organic matter (POM) included woody debris <10 cm in diameter or <1 m long.

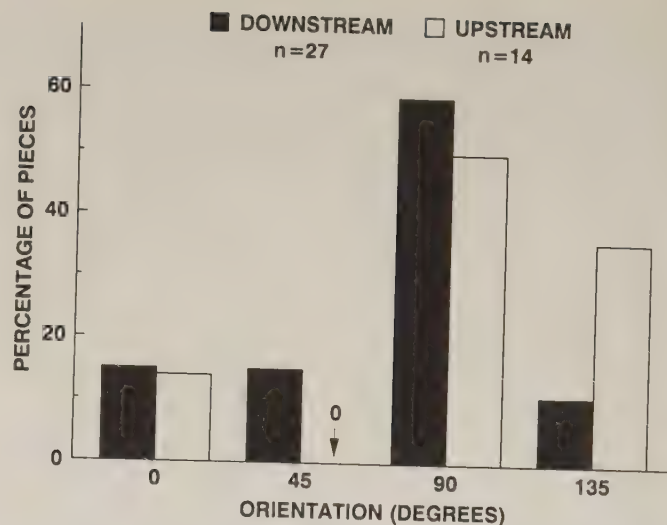


FIG. 3. Orientations of pieces of LWD that formed pools in four sections upstream and seven sections downstream from Marine Drive. Orientation classes consisted of 45° quadrants centered around the bearing shown, with 0° referring to debris with tips pointing straight upstream. Debris oriented in analogous, but opposite, quadrants (e.g. centered at 135 and 225°) was combined for each class.

estimated population was captured and weighed, except when the balance malfunctioned. Because on average about 80% of the fish in each section were captured on the first pass, and most of the rest retreated beneath cover, few fish probably escaped during the brief loss of the block net in section 6. If fish were lost, estimated numbers and biomass would be slightly less than, and capture probabilities slightly greater than, the true values for this section.

Cutthroat trout populations consisted primarily of fish <200 mm long (see also Northcote and Hartman 1988; Heggenes et al. 1991), except in the Cutthroat Creek section where 7 of 30 trout were >200 mm and none was smaller than 120 mm. All but two salmonids in this section were captured in a large deep pool formed at the outlet of a culvert, which had 2.7 m of wide undercut bank. Four of the largest trout bore finclips or scars from tags probably applied when the fish were captured in Musqueam Park by previous investigators (Heggenes et al. 1991), which suggests that these fish may have been anadromous. Upstream movement of cutthroat was probably limited by the 31-m culvert because calculated velocities were >1.2 m·s⁻¹ at depths ≥15 cm, and the outlet dropped approximately 30 cm at low flow (Fausch 1990).

Standing crop of age 1+ and older salmonids was low (10–25 kg·ha⁻¹) in the three simple sections and much higher (93–200 kg·ha⁻¹) in the four complex sections ($P < 0.02$ by t -test for unequal variance). Although the simple sections held small numbers of age 1+ cutthroat (estimated 70–125 mm from length-frequency histograms) and coho presmolts and relatively large numbers of age 0+ coho (K. D. Fausch, pers. obs.), no older cutthroat (>125 mm) were captured there. Mean weights (grams) of age 1+ and older cutthroat trout and coho salmon were significantly greater in complex than in simple sections (cutthroat (mean (SE)), 48.5 (6.27) versus 10.4 (1.03), $P = 0.0001$; coho, 10.2 (0.34) versus 8.5 (0.38), $P = 0.0012$ by t -test).

Relationships among Fish Biomass and Habitat

Pool volume, section volume, mean depth, and length of overhead cover were all strongly and significantly intercorre-

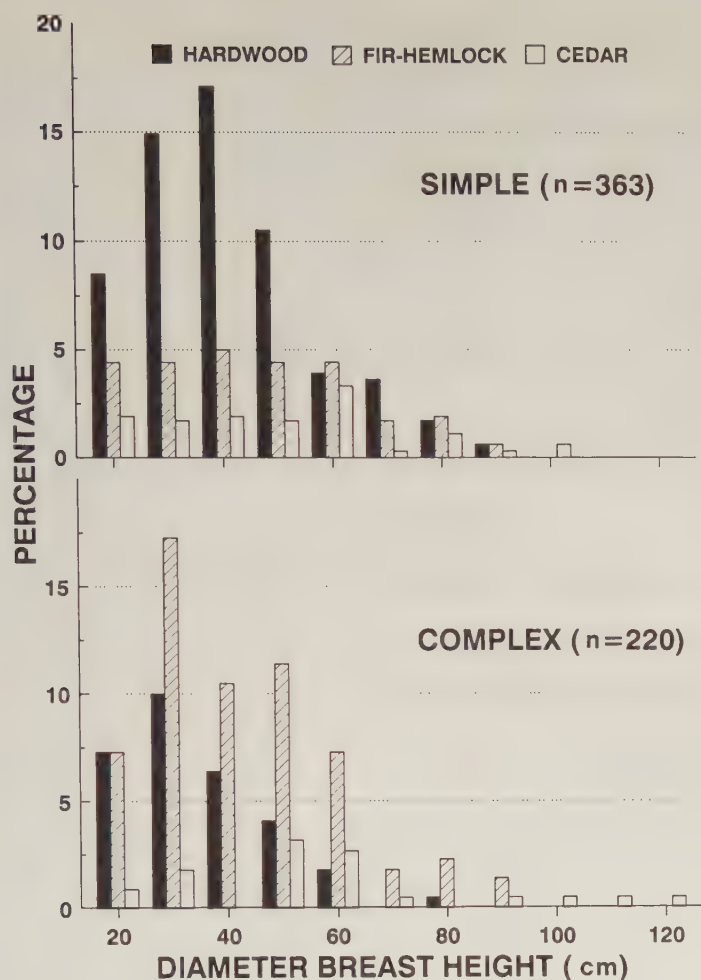


FIG. 4. Distribution of DBH of all trees ≥ 20 cm DBH within 30 m either side of Musqueam Creek in Musqueam Park, adjacent to the 332-m simple reach and the 209-m complex reach. Hardwoods include predominantly red alder, but also broadleaf maple (*Acer macrophyllum*) and a few western white birch (*Betula papyrifera*). The total number of trees measured adjacent to each reach is shown.

lated ($r = 0.79\text{--}0.97$, $P < 0.05$ for six pairs) for the seven sections downstream from Marine Drive where fish were sampled, which reflects the strong association between these variables and LWD. Because variance in biomass of age 1+ and older salmonids increased at higher values of these habitat variables (e.g. Fig. 5), we calculated correlation and regression coefficients after transforming both biomass and habitat variables using logarithms. Less stringent transformations were not sufficient to stabilize variance.

Total biomass of age 1+ and older salmonids was strongly correlated with all four habitat variables ($r = 0.85\text{--}0.96$, $p < 0.01$), but was most strongly associated with pool volume and depth ($r = 0.96$, $P < 0.01$ for both). Coho biomass was most strongly associated with overhead cover ($r = 0.92$, $P < 0.01$) and cutthroat biomass with mean depth ($r = 0.93$, $P < 0.01$), although both were also strongly correlated with pool volume ($r = 0.88$ and 0.90 , $P < 0.01$).

Because correlations between salmonid biomass and pool volume were most consistent among species, we calculated the following linear model after transforming the data:

$$\begin{aligned} \text{Log}_{10} \text{ salmonid biomass (kg)} = & \\ & -0.7044 + 0.9687 \log_{10} \text{ pool volume (m}^3\text{)} \\ & (r^2 = 0.92, P = 0.0006, \\ & \text{SE}_{\text{intercept}} = 0.0882, \text{SE}_{\text{slope}} = 0.1268). \end{aligned}$$

The error in measuring pool volume was small (estimated $< 0.25 \text{ m}^3$), so no correction was made for possible bias in the slope (Snedecor and Cochran 1967).

It is possible that a few large cutthroat captured in Cutthroat Creek were anadromous fish unable to ascend the culvert, although Heggenes et al. (1991) found that few trout in Musqueam Park moved more than 50 m during winter through late summer. This may, however, partially account for the higher biomass in section 11 compared with others with complex habitat (Fig. 5).

The relationship between biomass and pool volume is nearly linear because the exponent of the power function we fit (equivalent to the slope after logarithmic transformation) is close to 1. This assumption seems biologically reasonable, although we have no data for sections with intermediate pool volume to substantiate it. Finally, we caution against using this equation to predict salmonid biomass in other similar streams, or in this stream during other years, without validating it (cf. Fausch et al. 1988).

Discussion

Our results indicate that LWD in Musqueam Creek plays a critical role in creating and maintaining pools which, in turn, provide important habitat for salmonids. Sections that had been cleaned of woody debris had little pool volume (Table 1) and harbored few age 1+ and older salmonids (Table 4) whereas those where debris was left had much greater pool volume and salmonid biomass (Fig. 5).

Large Woody Debris and Pool Formation

Recent research has established the importance of LWD in shaping channel morphology and forming pools in Pacific Northwest streams (Bisson et al. 1987; Bilby and Ward 1989; Robison and Beschta 1990a, 1990b). These studies indicate that most pools are formed by LWD (Andrus et al. 1988) and that debris removal triggers changes that lead to wide, shallow channels with little pool volume at low flows (Bilty 1984; Bisson and Sedell 1984; Heifetz et al. 1986), similar to those we found in the cleaned reach of Musqueam Creek.

We found clear differences between simple and complex sections in sinuosity, width, depth, pool volume, and overhead cover for fish (Table 1), but differences in LWD were more subtle. Total LWD abundance, volume, and biomass within the bankfull channel were variable and the range of values generally similar between simple and complex reaches, although most values were lower than for undisturbed PSP reaches (Table 2). Similarly, when only debris that influenced channel morphology and flow during bankfull or lower discharge was included, LWD volume was variable and similar in simple and complex sections.

However, the clearest difference was that complex sections (except section 2) had more pieces of debris that influenced channel morphology than simple sections. This is perhaps also the best measure of "effective" debris because small streams are incapable of moving pieces of even moderate diameter (approximately 30 cm) if they are long enough to be stable (Keller and Swanson 1979; Bilby and Wasserman 1989; Robison and Beschta 1990a, 1990b), making debris volume less relevant. Moreover, relatively few pieces of LWD in each complex section accounted for most of the pool volume. Of the 6–11 pools in each of the four complex sections, the two largest in each made up 46–75% of pool volume. All were formed by

TABLE 4. Population estimates for age 1+ and older coho salmon and cutthroat trout in seven simple and complex sections of Musqueam and Cutthroat creeks during 5–12 April 1990. Estimates of population size ($\hat{N}$) and probabilities of capture ($\hat{p}$) were calculated using the generalized removal estimator in the program CAPTURE (White et al. 1982). In all cases, the number of fish captured in all passes was used as the lower 95% confidence limit (CI) because it exceeded the calculated lower limit.

Section	Species	$\hat{p}$	$\hat{N}$ (CI)	Biomass (g) ($\pm$ CI)	Standing crop (kg·ha ⁻¹)
<i>Simple</i>					
3	Coho	0.78	29 (29–30)	233 ^a	16.3
	Cutthroat	0.77	11 (11–12)	118 ^a	8.3
	Total			350 ^a	24.6
4	Coho	0.75	12 (12–13) ^b	104 $\pm$ 9	9.9
	Cutthroat	0.89	8 (8–8)	105 $\pm$ 3	10.1
	Total			209 $\pm$ 12	20.0
5	Coho	0.60	3 (3–5) ^b	39 $\pm$ 18	3.1
	Cutthroat	0.69	11 (11–13) ^b	90 $\pm$ 13	7.1
	Total			129 $\pm$ 31	10.1
<i>Complex</i>					
1	Coho	0.82, 0.58 ^c	75 (75–78)	693 $\pm$ 30	55.9
	Cutthroat	0.80, 0.75 ^c	15 (15–15)	456 $\pm$ 11	36.8
	Total			1149 $\pm$ 41	92.7
2	Coho	0.75, 0.71 ^c	60 (60–61)	479 ^a	38.0
	Cutthroat	0.72, 0.78 ^c	25 (25–26)	791 ^a	62.9
	Total			1270 ^a	100.9
6	Coho	0.87	27 (27–28)	410 $\pm$ 8	41.9
	Cutthroat	0.95	22 (22–22)	1260 $\pm$ 5	128.7
	Total			1671 $\pm$ 13	170.6
11	Coho	0.89	17 (17–17) ^b	249 $\pm$ 4	22.6
	Cutthroat	0.86	30 (30–31)	1954 $\pm$ 34	177.2
	Total			2203 $\pm$ 38	199.7

^aWeights of some (section 2) or all (section 3) fish were estimated by species-specific length–weight regressions based on fish captured in all sections, so no standard errors for biomass could be calculated.

^bThe chi-square test in CAPTURE detected declining capture probability after the first pass ($P < 0.20$), so these three-pass population estimates and confidence intervals probably underestimate the true population size slightly (Riley and Fausch 1992). However, in all cases, either no fish were captured on the third pass or no fish were captured on the second pass and only one on the third pass, so most fish were probably captured.

^cFour passes were completed for sections 1 and 2, which allows calculating a different capture probability for the first pass versus subsequent passes and produces a more accurate estimate.

LWD or tree roots >10 cm in diameter, except in section 6 where both were formed by scour beneath undercut banks, in one case combined with a plunge pool at the culvert outlet. Andrus et al. (1988) also found that pools in a coastal Oregon stream were formed by relatively few influential pieces of debris and proposed that beyond some threshold, factors other than the abundance of debris control pool formation. We cannot explain the lack of debris in section 2, although we suspect that the channel may have been formed by previous debris, now rotted away or incorporated into the bed or banks.

Our analysis of pool-forming agents also highlights the importance of LWD. Most pools, and most pool volume, in reaches throughout the Musqueam Creek system were scour or plunge pools formed by LWD or large tree roots (Table 3; see also Bilby and Ward 1989). Heifetz et al. (1986) and Andrus et al. (1988) also reported that 70–75% of pools were formed by LWD. Of debris that formed pools, most was oriented either perpendicular to the flow or angled downstream (Fig. 3), a conclusion also reached by Bilby and Ward (1989) for streams of similar size in foothills of the Cascade Mountains in Oregon.

Natural distributions of LWD in small streams may be skewed toward perpendicular because trees rooted farther away from channels can intersect them only in this orientation (cf. Robison and Beschta 1990a).

Large Woody Debris Volume

Bankfull channel debris volume and biomass were generally low in Musqueam Park (Table 2). Most of the volume was contributed by one large piece in six of nine sections where it was calculated. In contrast, the average bankfull biomass for sections in the PSP reach was similar to values measured in similar biomes. Harmon et al. (1986) reported biomass averaging 270 Mg·ha⁻¹ (SE = 53, maximum 670 Mg·ha⁻¹) for nine streams 11–20 m wide in Sitka spruce (*Picea sitchensis*) and western hemlock forests of coastal British Columbia and averages of 252 Mg·ha⁻¹ (SE = 25, maximum 550 Mg·ha⁻¹) for 25 streams <10 m wide in Douglas-fir forests of Oregon's Cascade Mountains, both based on the same wood density we used.

We suspect that debris volumes are low in Musqueam Park because the small amount of standing or fallen debris that might

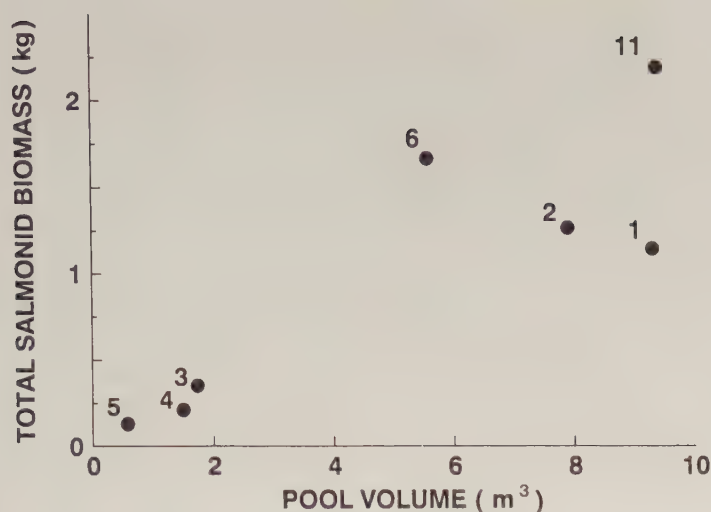


FIG. 5. Total biomass of age 1+ and older salmonids as a function of pool volume for seven sections of Musqueam (sections 1–6) and Cutthroat (section 11) creeks sampled in April 1990 (see Fig. 1). Ninety-five percent confidence intervals for biomass are smaller than the points in each case and are given in Table 4.

enter the stream is periodically removed for safety or aesthetic reasons, even that adjacent to complex sections. As a result, the framework of debris left from the preharvest stand that forms pools and binds habitat together has largely rotted and broken into small pieces (Fig. 2) and soon will collapse and wash away. Other investigators predict that LWD reaches a minimum approximately 100 yr after clearcut in Pacific Northwest streams (Harmon et al. 1986; Murphy and Koski 1989), but even this amount probably far exceeds the LWD left in Musqueam Creek after cleaning. When the remnants of original debris wash away, pool volume will be at its lowest ebb, so debris inputs or other habitat enhancement measures (e.g. Ward and Slaney 1979) are needed now if habitat for age 1+ and older cutthroat and overwintering juvenile coho is to be maintained.

It is also clear that the lack of debris in simple sections is not due primarily to differences in riparian sources. Although conifers are relatively more abundant adjacent to the complex reach (Fig. 4), crude estimates indicate that debris would be more abundant in both simple and complex reaches if none had been removed. A more sophisticated model of future debris inputs is available (Van Sickle and Gregory 1990) but is beyond the scope of this study.

Pools as Salmonid Habitat

Although previous research has shown a direct link between LWD and channel morphology, our knowledge of the importance of pools to salmonids is less complete. Several investigators proposed that pool volume and overhead cover, both formed by debris, provide important overwinter habitat for juvenile coho salmon (Hartman 1965; Bustard and Narver 1975a; Bisson et al. 1985, 1988) and cutthroat trout (Glova 1986). Both have been implicated in models that predict coho salmon abundance or biomass from pool and/or debris volume (Nickelson et al. 1979; Tschaplinski and Hartman 1983; Murphy et al. 1986). Experiments in laboratory (Hartman 1965) and artificial channels (Bustard and Narver 1975b; McMahon and Hartman 1989) also showed the importance of pools with complex habitat to overwintering coho salmon.

Despite these field observations and small-scale experiments, few investigators have conducted field experiments to measure salmonid populations in sections where debris was removed versus control reaches. Moreover, in all such experiments know to us the treatment was a combination of both canopy and debris removal. For example, Bisson and Sedell (1984) and Murphy et al. (1985) reported that salmonid biomass was higher in sections adjacent to clearcuts that had been cleaned of logging slash than in those next to old-growth, but effects of reduced canopy on light, temperature, and primary and secondary production were inseparable from the effects of debris removal on channel morphology (cf. Murphy et al. 1981, 1986; Hawkins et al. 1983). In both cases, the clearcut sections produced primarily age 0 salmonids due to known or suspected higher primary and secondary production, but harbored few age 1+ and older parr due to lack of pools with complex cover. Murphy et al. (1985, 1986) found that reaches where buffer strips were left adjacent to clearcuts, which often had more debris and pool volume, held more coho and steelhead parr than sections adjacent to clearcuts where some LWD generally had been removed from the channel.

We also found that age 1+ and older cutthroat and coho psmolts were larger and more abundant in sections with complex habitat (Table 4), which supports the hypothesis that more salmonid smolts are produced from such habitat. Moreover, although many age 0 coho were captured in three sections with simple habitat, none held fish older than age 1+. Tschaplinski and Hartman (1983) reported that during the first autumn freshet, most age 0 coho emigrated from sections in Carnation Creek that had little LWD (see also Bisson et al. 1985; Johnson et al. 1986; Murphy et al. 1986), which provides a mechanism to explain these results. Pools formed by LWD also provided the only habitat capable of supporting age 2+ and older cutthroat trout during extreme low flows of late summer (K. D. Fausch and T. G. Northcote, pers. obs.).

Although we chose sections to contrast the effects of long-term debris removal in Musqueam Creek, we were unable to fully randomize treatments to sections or to employ a paired design. The most important consequence is that we cannot rule out the possibility that difference in some other environmental factor, such as gradient, caused the lower biomass in the mid-reach of Musqueam Park that was cleaned. This lack of randomly assigning treatments to sections is a form of pseudoreplication (Hurlbert 1984). The overriding effect of some other factor seems unlikely, however, given the overall similarity in gradient and other feature among sections. Moreover, the complex sections were interspersed upstream and downstream of the simple reach and in a tributary, and we made conservative statistical inferences, both of which strengthen our conclusions that salmonid habitat and biomass were greater in complex sections.

Salmonid Production Forgone

The outcome of cleaning woody debris from stream channels is that much fish habitat is lost as channel morphology becomes simple, and much fish production is subsequently forgone. The overall effects are likely to be more drastic after relatively complete debris removal, such as occurred in Musqueam Creek, compared with removing only new slash and debris as is currently practiced after logging (Bisson et al. 1987). Even the latter can cause modest but significant declines in salmonid production (Dolloff 1986; Elliott 1986) which may, however, be ameliorated by increased stream temperature and light (Hawkins et al. 1983; Bisson and Sedell 1984; Hartman et al. 1987).

Our data allow us to make a preliminary estimate of the salmonid production forgone during 1990 in the 332-m reach of Musqueam Park with simple habitat, as a result of prior stream cleaning. Based on average conditions in the four complex sections, one would expect this simple reach to be 26% longer due to increased sinuosity created by LWD (Table 1) and to contain 0.13 m³ of pool volume per metre of stream. The resulting 55.0 m³ of pools would have produced 9.6 kg of salmonid biomass (age 1+ and older), if our model is accurate. This exceeds the 1.6 kg of biomass estimated for the simple reach, based on similar assumptions about current conditions, by 5.0 times.

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Production and Vertical Migration of *Ceratium hirundinella* in Relation to Phosphorus Availability in Eau Galle Reservoir, Wisconsin

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Seasonal production of *Ceratium hirundinella* and its diel migratory patterns were examined in relation to phosphorus (P) availability in eutrophic Eau Galle Reservoir, Wisconsin (USA). During mid-June, hypolimnetic P gradients ($0.030\text{--}1.045\text{ mg}\cdot\text{L}^{-1}$) developed as internal P loading was high ($14.7\text{--}18.0\text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). *Ceratium* migrated as much as 4 m into the upper hypolimnion at night. Subsequent increases in *Ceratium* biomass, gross primary productivity, and chlorophyll *a* indicated retrieval of hypolimnetic P for production. During early July, anoxia restricted vertical migration of *Ceratium* into the hypolimnion. Surplus cellular P was low during this period, while alkaline phosphatase activity increased to a maximum, suggesting P limitation of *Ceratium* production. During late July and August, P-rich interflows from the Eau Galle River entered the reservoir at the base of the epilimnion. *Ceratium* migrated into these interflows at night, with corresponding increases and decreases in surplus cellular P and alkaline phosphatase activity, respectively. *Ceratium* production increased to a maximum in early September, following these periods of high external P input. These results directly support the hypothesis that *Ceratium* can access multiple P sources through vertical migration.

La production saisonnière et les régimes migratoires circadiens de *Ceratium hirundinella* ont été examinés en fonction de la disponibilité du phosphore (P) dans le réservoir Eau Galle, au Wisconsin (États-Unis). À la mi-juin, des gradients hypolimnétiques de P ($0,030\text{--}1,045\text{ mg}\cdot\text{L}^{-1}$) sont apparus car la charge interne de P était élevée ($14,7\text{--}18,0\text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). Les *Ceratium* se déplaçaient la nuit jusqu'à 4 m dans l'hypolimnion supérieur. Les augmentations ultérieures de la biomasse des *Ceratium*, de la productivité primaire brute et de la teneur en chlorophylle *a* indiquaient l'utilisation du P hypolimnétique à des fins de production. Au début de juillet, l'anoxie limitait la migration verticale des *Ceratium* à l'hypolimnion. Les excédents de P cellulaire étaient faibles pendant cette période, mais l'activité de la phosphatase alcaline atteignait un maximum, ce qui porte à croire à une limitation par le P de la production des *Ceratium*. Pendant la fin de juillet et en août, des courants sous-jacents riches en P provenant de la rivière Eau Galle pénétraient dans le réservoir à la base de l'épilimnion. Les *Ceratium* migraient dans ces courants pendant la nuit et l'on notait une augmentation du P cellulaire excédentaire et une baisse de l'activité de la phosphatase alcaline. La production des *Ceratium* augmentait pour atteindre un maximum au début de septembre, suite à ces périodes d'importants influx de P externe. Ces résultats appuient directement l'hypothèse selon laquelle les *Ceratium* peuvent avoir accès à diverses sources de P de par leurs migrations verticales.

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Considerable attention has been given to physical mechanisms responsible for the transport of hypolimnetic phosphorus (P) to the euphotic zone for phytoplankton production (Stauffer and Lee 1973; Imboden and Emerson 1978; Larson et al. 1981; Stefan and Hanson 1981; Kortmann et al. 1982; Wodka et al. 1983; Stauffer and Armstrong 1984; Stauffer 1985, 1987). In addition, direct retrieval of P from the hypolimnion by motile forms of phytoplankton (Mitchell and Galland 1981; Salonen et al. 1984; Osgood 1988; Taylor et al. 1988) may provide an important alternative mechanism of P transport. In particular, the dinoflagellate *Ceratium hirundinella* has been shown to migrate on a diel basis (Talling 1971; Heaney 1976; Heaney and Talling 1980; Taylor et al. 1988), often through steep temperature gradients (Frempong 1984).

While this capability by *Ceratium* clearly provides an opportunity to obtain hypolimnetic P in lakes, very little direct evidence exists to link its production to access to chemical gradients. In addition, this migratory behavior in reservoirs may provide access to P from interflowing river water (Taylor et al. 1988).

In Eau Galle Reservoir, Wisconsin (USA), we have expanded earlier information (Taylor et al. 1988) by examining *Ceratium*'s production and vertical migration patterns in relation to riverine inputs and internal P loading from profundal sediments. The reservoir is small (0.6 km^2), eutrophic (see Barko et al. 1990), and exhibits hypolimnetic anoxia and high internal P loading from both profundal (mean = $8.0\text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$; James et al. 1991) and littoral (mean = $3.6\text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$;

James and Barko 1991) sediments during the summer. P-rich storm inflows typically enter the reservoir within the metalimnion at about the 2- to 4-m depth during the summer (Gunkel et al. 1984). Discharges from the reservoir occur via an uncontrolled surface withdrawal structure and a controlled hypolimnetic withdrawal gate. This reservoir provides exceptional conditions for evaluating the migratory and nutritional behavior of this species because *Ceratium* contributes more biomass than the remainder of the combined phytoplankton community during the summer (Barko et al. 1990).

Methods

Limnological Determinations

Water samples were collected weekly in Eau Galle Reservoir at a deep (9 m), centrally located station from June through mid-September 1987. Temperature and dissolved oxygen were measured at 0.25- to 0.5-m intervals with a Hydrolab Surveyor II calibrated against a NBS thermometer and Winkler determinations (APHA 1985). Water samples for total P (TP) and total soluble P (TSP) were collected at 0.25-m intervals from the reservoir surface to the 3-m depth and over the same intervals from 7 m to the reservoir bottom. Samples over intermediate depths (3–7 m) were collected at 0.5- to 1-m intervals. A peristaltic pump (Masterflex) equipped with small-diameter tubing (6 mm inside diameter) was used to collect water samples over the depth range of 0–7 m. A pneumatically driven, close-interval syringe sampler (James et al. 1990) was used to collect samples between the 7- and 9-m depths. Samples for TSP obtained by pumping were filtered immediately (0.45- μm membrane, Nalge) in syringes to prevent exposure to air. TSP samples collected from close-interval syringe samplers were filtered in situ through 0.45- μm membrane filters attached to each syringe. TP and TSP were determined colorimetrically on a Technicon Auto-Analyzer II following persulfate oxidation (APHA 1985). The detection limit for all P analyses was $5 \mu\text{g}\cdot\text{L}^{-1}$.

River water samples for TP and TSP analyses were collected weekly at fixed stations located on the minor tributaries of Eau Galle Reservoir (Lohn, Lousey, and French creeks). Samples from the Eau Galle River and the tailwater were with few exceptions collected every day. During all periods of high inflow, samples were collected on the Eau Galle River at hourly intervals using an automated sampler (ISCO). Reservoir volume and flows from tributaries and the tailwater were determined either from continuous recording gauges or crest gauge recorders.

Budgetary Determinations

Daily external TP loading and TP discharge were determined by multiplying the flow rate by the TP concentration of the flows. Internal TP loading was calculated by mass balance according to the following equation: change in TP mass = (external TP load – TP discharge) + internal TP load. TP mass was calculated as the sum of volume-weighted TP in the water column. TP mass was also integrated over the 0- to 3-m depth interval (i.e. epilimnion).

The daily residence time of the epilimnion was estimated as the water volume of the reservoir between 0 and 3 m divided by discharge from the reservoir. The upper 3 m of the water column was chosen to represent an average epilimnetic depth, based on 4 yr of previous water temperature data collected for the period June–August (mean depth = 3.07 m; W. F. James,

unpubl. data). The position of river water entry into the reservoir (i.e. interflow, overflow, or underflow) was estimated by comparing water temperatures in the well-mixed Eau Galle River with reservoir water column temperatures ($\pm 1^\circ\text{C}$). Past dye studies have indicated that Eau Galle River water enters the reservoir as interflows within strata of equal density, based on temperature.

Phosphorus Limitation Assays

Three replicate water samples were collected weekly at the depth of maximum daytime chlorophyll fluorescence (see below) for determination of surplus cellular P and alkaline phosphatase activity, both of which were normalized with respect to seston dry mass (Fitzgerald and Nelson 1966; APHA 1985). Microscopic cell counts of samples collected at fluorescence peaks consistently showed *Ceratium* accounting for >95% of the algal biomass.

Surplus cellular P was estimated as hot-water-extractable P (HEP) using the method of Fitzgerald and Nelson (1966). Seston collected on glass fiber filters (Gelman A-E) was placed in a water bath at 100°C and boiled for 1 h and then filtered through a 0.45- μm membrane and analyzed colorimetrically for soluble reactive P using the ascorbic acid method (APHA 1985).

Alkaline phosphatase activity was estimated as the rate of hydrolysis of nonfluorescent 3-*o*-methylfluorescein phosphate (MFP) to fluorescent methylfluorescein (MF) in accordance with the methods of Perry (1972) as modified by Wetzel (1981). The rate of hydrolysis to MF was measured as the increase in fluorescence (5- to 30-min intervals) on a Sequoia Turner fluorometer (model 111) over a maximum of 2 h. Simultaneous blanks were measured to correct for background fluorescence. The increase in fluorescence was linear with time for all analyses. Fluorescence measurements were converted to concentration units (micromoles PO_4 per litre) and regressed against time to calculate alkaline phosphatase activity (micromoles PO_4 per litre per minute).

Phytoplankton Production and Vertical Migration

Water samples integrated between the reservoir surface and the 3-m depth were collected each week with an integral water sampler (Barko et al. 1984) for phytoplankton enumeration and chlorophyll *a* determination. Subsamples for phytoplankton enumeration were preserved with acid Lugol's solution (Vollenweider 1969). Taxonomic identification and enumeration of phytoplankton was accomplished in accordance with the procedures of Lund et al. (1958). Cell volume for individual species was computed from average cell dimensions (Munawar and Munawar 1976). Phytoplankton biomass is reported as grams fresh weight per cubic metre, assuming a cellular specific gravity of $1.0 \text{ g}\cdot\text{mL}^{-1}$ (Nauwerck 1963). Chlorophyll *a*, corrected for phaeopigments, was determined spectrophotometrically after extraction with 90% alkaline acetone (APHA 1985).

Gross primary productivity (GPP) was estimated from dissolved oxygen changes in 300-mL light and dark bottles (APHA 1985) incubated at 0.5-m intervals from the reservoir surface to the 3-m depth. Bottle incubation periods (2–3 h) overlapped solar noon. GPP was converted to carbon uptake assuming a photosynthetic quotient of 1.2 (Wetzel and Likens 1979). GPP was volume-weighted to obtain an integral production rate for the upper 3 m of the reservoir.

Chlorophyll fluorescence was determined at 0.25-m intervals from the reservoir surface to various depths using a peristaltic

pump and Turner Designs fluorometer (model 10-005R). Measurements were made during the day (11:00–13:00) at weekly intervals and during the night (00:00–02:00) at biweekly intervals. Fluorescence values were converted to relative units (percentage) by dividing fluorescence measurements at each depth by the maximum fluorescence over all depths during each sampling period. There was a strong correlation between relative *Ceratium* cell numbers and fluorescence within a given vertical profile ($r^2 > 0.95$; $p < 0.05$). Therefore, in situ fluorescence was used as a simple surrogate to track *Ceratium* population migrations. Photosynthetically active radiation (PAR) was measured at 1-m intervals on each sampling date with a cosine Licor quantum radiometer (model LI-185B).

Results

Phytoplankton Production in Relation to Hydrologic Events and Phosphorus Supply

During periods of nominal inflow from the Eau Galle River (e.g. early June; Fig. 1a), 50–100% of the discharge from the reservoir occurred through the hypolimnetic withdrawal gate. However, during periods of high inflow in late July and mid-

August, 70–90% of the discharge occurred through the surface withdrawal structure, due to increases in reservoir pool elevation above the surface withdrawal structure. The hydraulic residence time of the epilimnion (Fig. 1a), usually >50 d during nominal inflows and hypolimnetic withdrawal, decreased during high inflows and surface withdrawal to <7 d in late July and to <3 d in mid-August.

Chlorophyll *a* and GPP exhibited major peaks in early July and early September (Fig. 1b). Major peaks in GPP occurred approximately 1 wk prior to major peaks in chlorophyll *a*. Seasonal variations in phytoplankton biomass (Fig. 1c) coincided closely with variations in chlorophyll *a* (Fig. 1b). *Ceratium* dominated the phytoplankton assemblage on all dates except 25 August, accounting for 36–94% (mean = $70\% \pm 6.0$) of total biomass during the summer (Fig. 1c).

The increase in *Ceratium* biomass from June to mid-July (Fig. 1c) coincided closely with increases in both TP mass in the epilimnion (Fig. 1d) and internal TP loading (Fig. 1e). Strong vertical gradients in TP developed above the profundal sediment during this period (Fig. 1f), coincident with the occurrence of anoxia in the hypolimnion (see Fig. 3). In early July, a TP peak in the epilimnion occurred between the 1- and

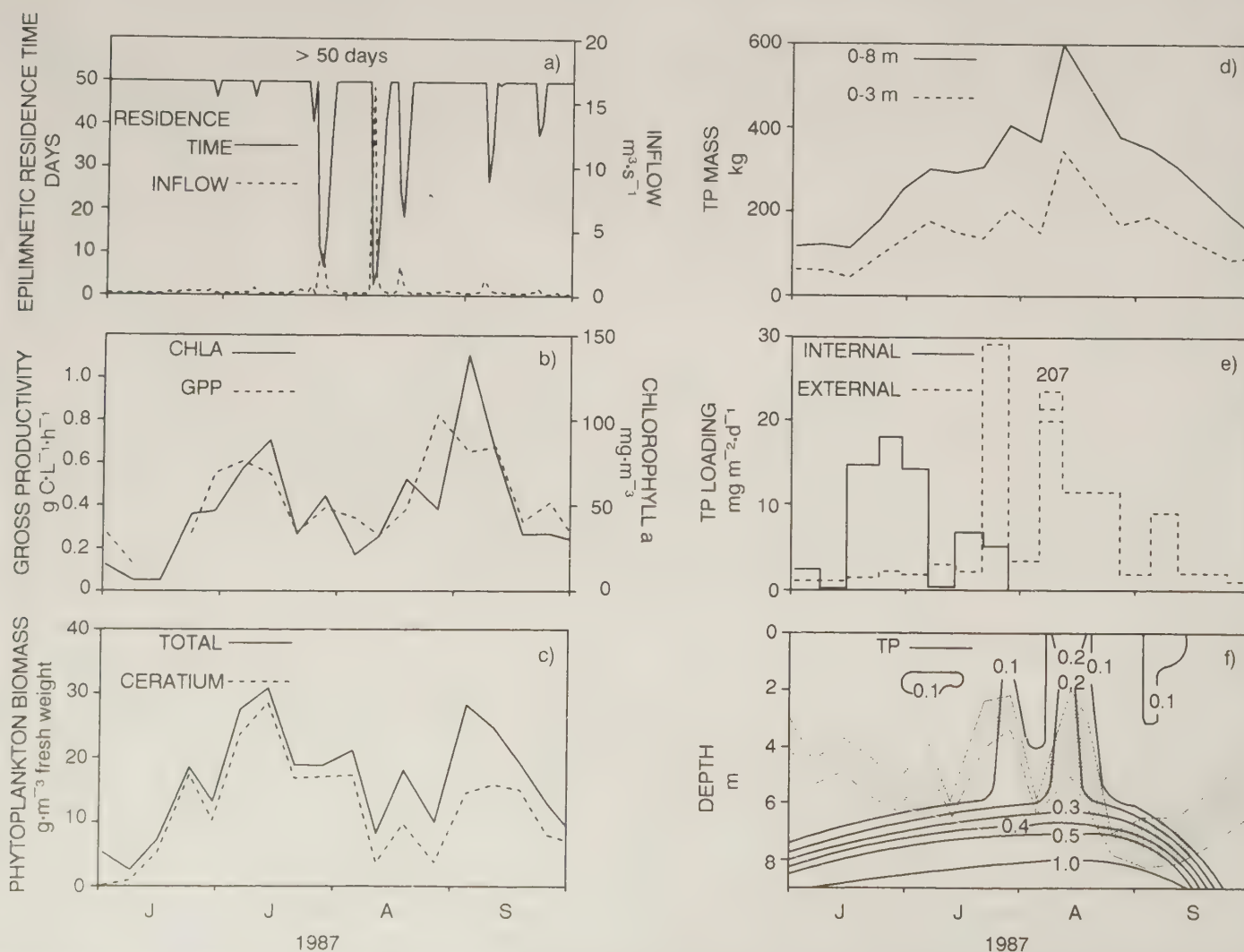


FIG. 1. Seasonal variations in the (a) hydrograph of the Eau Galle River and epilimnetic residence time, (b) gross primary productivity and chlorophyll *a*, (c) total phytoplankton and *Ceratium* biomass, (d) TP mass between 0–8 and 0–3 m, (e) internal and external TP loading, and (f) vertical and seasonal variations in TP concentration ($\text{mg} \cdot \text{L}^{-1}$). The shaded area represents the estimated region of riverine interflow based on water temperature comparisons between the Eau Galle River and the reservoir water column.

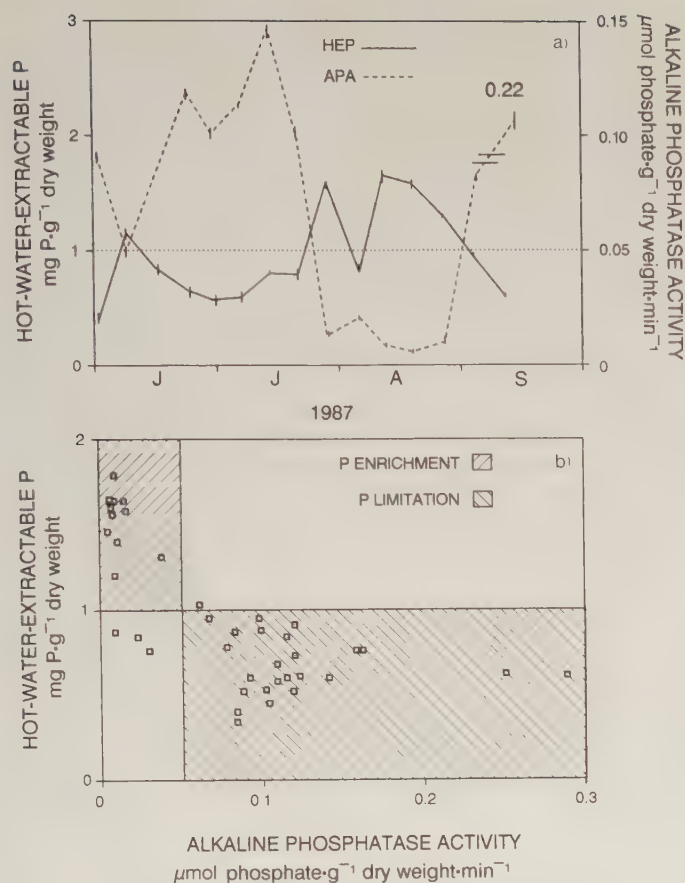


FIG. 2. (a) Seasonal variations in mean HEP (surplus cellular P) and alkaline phosphatase activity (± 1 SE) in the upper 3 m of the water column. The horizontal dotted line represents P limitation threshold levels. HEP above and alkaline phosphatase activity below this line are considered potentially P limiting. (b) Relationship between HEP and alkaline phosphatase activity. The hatched areas represent regions of potential P limitation or P enrichment.

2-m depths (Fig. 1f), in association with the peak in *Ceratum* biomass (Fig. 1c).

Sharp declines in *Ceratum* biomass in August (Fig. 1c) resulted from cellular discharge during high inflows and corresponding decreases in the hydraulic residence time of the epilimnion (Fig. 1a). During these storms in August, TP mass in both the reservoir and epilimnion increased greatly (Fig. 1d) due to high external P loading (Fig. 1e). Increases in TP occurred at depths in the water column that coincided with the estimated position of interflows (Fig. 1f). A second major peak in *Ceratum* biomass occurred in early September (Fig. 1c), following these periods of high external TP loading in August (Fig. 1e).

Alkaline phosphatase activity and HEP varied inversely with respect to one another throughout the summer (Fig. 2a). During the onset of *Ceratum* biomass increase in early June (Fig. 1c), HEP exhibited a peak, while alkaline phosphatase activity declined (Fig. 2a). With increased *Ceratum* production during June through mid-July (Fig. 1c), HEP declined steadily and alkaline phosphatase activity increased (Fig. 2a). During late July and August, HEP exhibited additional peaks, while alkaline phosphatase activity declined to a minimum, coincident with high external P loading. During the development of the second major *Ceratum* peak in September, HEP again declined, while alkaline phosphatase activity increased to a maximum.

HEP and alkaline phosphatase activity (APA) were related in a negative logarithmic fashion ($r^2 = 0.54$; $\ln \text{HEP} = -\ln \text{APA} (0.31) - 1.47$). Below an HEP concentration of about $1.0 \text{ mg P} \cdot \text{g}^{-1}$, alkaline phosphatase activity increased (Fig. 2b). In contrast, HEP increased in concentration when alkaline phosphatase activity was less than about $0.05 \mu\text{mol PO}_4 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ (Fig. 2b). These trends suggest threshold values of HEP ($< 1.0 \text{ mg P} \cdot \text{g}^{-1}$) and alkaline phosphatase activity ($> 0.05 \mu\text{mol PO}_4 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) below or above which P limitation of phytoplankton production occurred (Fig. 2a and 2b).

Vertical Migration of *Ceratum* in Relation to Phosphorus Supply

Early during the first period of increase in *Ceratum* biomass (16–20 June), the population was concentrated at 3 m during the day and then migrated at night to near the 6-m depth, but avoided anoxic water (Fig. 3). During the day on 30 June, a major fluorescence peak occurred at 1 m and a secondary fluorescence peak occurred at 2 m. During the night on this date, fluorescence peaks occurred at 4 and 5 m. The location of the fluorescence peaks at night on both 20 and 30 June occurred in the upper hypolimnion where TP and TSP were high (Fig. 3). TP peaks in the epilimnion on these same dates coincided precisely with locations of fluorescence peaks, suggesting that TP was associated with *Ceratum* biomass. In contrast, TSP was near detection limits (i.e. $< 5 \mu\text{g} \cdot \text{L}^{-1}$) at these same depths (Fig. 3).

Dissolved oxygen decreased substantially in the hypolimnion in late June, and anoxic conditions existed between 3 m and the reservoir bottom by 14 July (Fig. 3). Anoxia limited vertical migration of *Ceratum* to the 0- to 3-m stratum on this date, and fluorescence declined substantially below 3 m. A fluorescence peak occurred at 1.5 m during the day and at 2.5 m at night, well removed from hypolimnetic P (Fig. 3).

During periods of high external P loading in late July and August, relatively high TP and TSP concentrations occurred within regions of interflow (Fig. 3) located within the metalimnion. On 28 July, a fluorescence peak occurred within the upper 2 m of the water column during the day (Fig. 3). At night, the fluorescence peak was located at 2.75 m, in the region of interflow. On 11 August, the fluorescence peak was located near the reservoir surface during the day and at 3 m during the night, again within the region of interflow.

The reservoir was weakly stratified during the increase in *Ceratum* production in early September (Fig. 3). At that time the epilimnion had expanded to > 3 m, and dissolved oxygen was present down to the 7-m depth. Fluorescence exhibited a major peak at 0.5 m and a minor secondary peak at 6.0 m during the day on 2 September (Fig. 3). That night, fluorescence was uniformly high within the upper 3 m of the water column and declined steadily with increasing depth.

Discussion

Seasonal variations in HEP and alkaline phosphatase activity were good indicators of P limitation in *Ceratum*. The same negative logarithmic relationship between HEP and alkaline phosphatase activity found here was demonstrated by Pettersson (1980) and Gage and Gorham (1985) as well. The threshold level of $1.0 \text{ mg HEP} \cdot \text{g dry weight}^{-1}$ estimated for Eau Galle Reservoir was similar to that ($0.8 \text{ mg HEP} \cdot \text{g}^{-1}$) determined by Fitzgerald and Nelson (1966) for laboratory cultures of *Anabaena*. Using the P limitation criteria established for Eau Galle

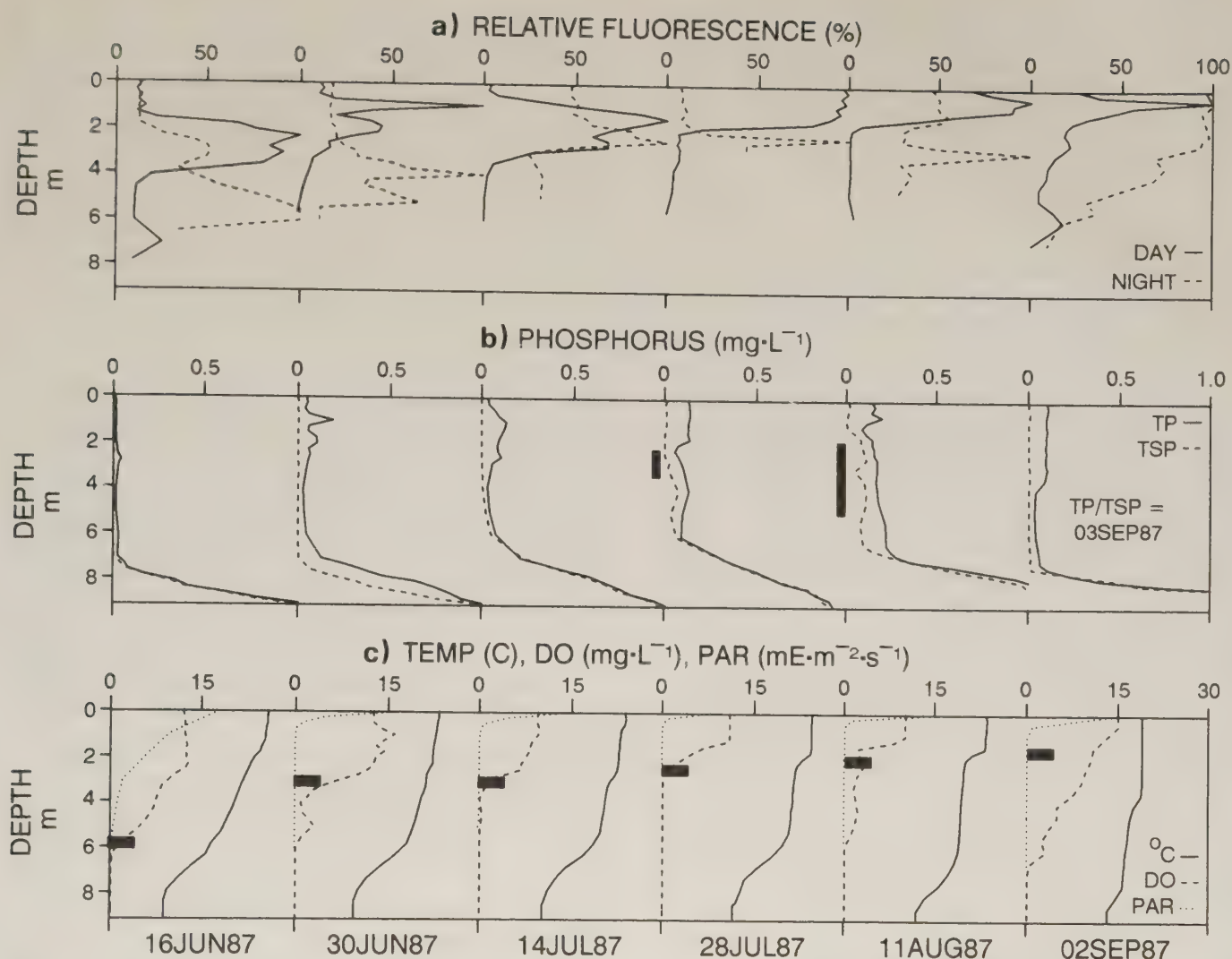


FIG. 3. Seasonal and vertical variations in (a) daytime and nighttime relative fluorescence, (b) TP and TSP, and (c) temperature, dissolved oxygen, and PAR. The vertical bars (Fig. 3b) represent the estimated regions of riverine inflow during storms. The horizontal bars (Fig. 3c) represent depths where relative light transmittance is $\leq 1\%$.

Reservoir, the phytoplankton community was apparently P limited throughout most of June and July whereas during periods of high external P loading to the reservoir (late July – August), the community was not P limited. P limitation occurred again in conjunction with increased phytoplankton production in early September.

During periods of increased phytoplankton production in June through mid-July, HEP declined markedly, indicating depletion of surplus cellular P reserves. External TP loading was minimal during this period; thus, internal mechanisms of TP flux to the epilimnion were apparently important in maintaining phytoplankton production. Internal P loading rates from profundal sediments were greater than $10 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ during June, creating strong vertical P gradients in the anoxic hypolimnion (i.e. Nürnberg 1984, 1987). However, transport of hypolimnetic P via physical processes, including vertical eddy diffusion (Jassby and Powell 1975; Imboden and Emerson 1978; Stefan and Hanson 1981) or wind-induced migrations of the metalimnion (James et al. 1990), was unlikely because TP gradients were not evident in the upper metalimnion (i.e. Stauffer 1985). Thus, vertical migration by *Ceratum* appeared to be the only plausible mechanism of nutrient retrieval from the hypolimnion.

Ceratum migrated as much as 4 m at night during June, as dissolved oxygen was in sufficient concentration to allow migration into the upper hypolimnion. Much of the hypolimnetic P was in a soluble form, presumably available for uptake by *Ceratum*. Finally, there was a strong correspondence between access to the hypolimnion in June and subsequent increases in *Ceratum* biomass, GPP, and TP mass in the epilimnion. These observations collectively suggest that *Ceratum* retrieved hypolimnetic P for its production in June.

As the *Ceratum* population increased in biomass, the region of anoxia expanded upward to a depth of 3 m in July. Nighttime respiratory activities of *Ceratum* at hypolimnetic depths may have contributed to dissolved oxygen depletion. Throughout the remaining summer, nighttime downward migrations by *Ceratum* were restricted by anoxia (i.e. Harris et al. 1979; Heaney and Talling 1980; Frempong 1984; Taylor et al. 1988). Thus, an important source of P was unavailable to the *Ceratum* population during mid- to late July. TSP concentrations were extremely low in the epilimnion and metalimnion at this time, possibly contributing to the decrease in *Ceratum* biomass occurring after mid-July.

High external P loadings provided an important source of P to the phytoplankton community during late July and August,

as P-rich interflows entered the reservoir at the base of the epilimnion. *Ceratium* migrated into these interflows at night, and its production did not appear to be P limited, as indicated by marked increases in HEP and declines in alkaline phosphatase activity. However, *Ceratium* biomass, rather than increasing as a result of P enrichment, decreased substantially due to cellular discharge from the reservoir during periods of high inflow, as nearly 90% of the reservoir discharge occurred through the surface withdrawal structure. Reported doubling times of 5–15 d for *Ceratium* during periods of exponential growth (Heller 1977; Elser and Smith 1985) could not have compensated for cellular losses during rapid turnover of the epilimnion (<3 d). *Ceratium* biomass rebounded in late August and early September, when the residence time of the epilimnion increased to >50 d.

These results demonstrate the effectiveness of *Ceratium* in obtaining P from multiple sources in Eau Galle Reservoir. Both the P-rich hypolimnion and interflows from the Eau Galle River serve as important sources of P retrieved by this migrating phytoplankton species. Littoral sources of P that are transported via nighttime convective circulation as interflow currents located at the base of the epilimnion (James and Barko 1991) are also within the migrational path of *Ceratium* during the summer in this reservoir. The ability of *Ceratium* to modify vertical migration patterns in the water column may confer a competitive advantage to this species, over nonmigrating species, when conditions of P limitation exist in the epilimnion and physical mechanisms of vertical P transport are absent.

Acknowledgments

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Statistical Interrelation of Length, Growth, and Scale Circulus Spacing: Use of Ossification to Detect Nongrowing Fish

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Estimates of growth over a short period are often difficult to obtain from fish unless individuals are tagged or experimental conditions are highly controlled. We demonstrate that the outer edge of scales (the osseoid layer) in tilapia (*Oreochromis* sp.) can be used to recognize nongrowing fish. We measured the width of the osseoid tissue at various positions of the anterior edge of the scale as well as inward to the first circulus on the edge of the scale in fed and starved fish. Our results indicated that response of the osseoid layer to growth rates occurred in 1 wk or less. Discriminant analysis based solely on osseoid variables classified over 84% of all fish correctly on the basis of the feeding treatment after 8 d of growth. This technique was field-tested in cage-cultured tilapia in Java, Indonesia. We were able to classify correctly 86% of the growing and nongrowing fish in the population after a 21-d period. These measurements can supplement the information collected from the spacing of circuli on the calcified portion of the scale to completely specify the recent growth history of individual fish.

Il est souvent difficile d'obtenir, en une courte période, des estimations de la croissance des poissons à moins de procéder au marquage d'individus ou de disposer de conditions expérimentales très contrôlées. Nous démontrons que la bordure extérieure des écailles (couche ossoïde) des espèces du tilapia (*Oreochromis* sp.) peut servir à déterminer si un poisson n'est pas en croissance. Nous avons mesuré, chez des poissons alimentés et non alimentés, la largeur des tissus ossoïdes en divers points de la bordure antérieure des écailles de même que, vers l'intérieur, au jusqu'au premier anneau de croissance en bordure de l'écaille. Les résultats obtenus montrent que la couche ossoïde réagit au taux de croissance en moins de 1 sem. Une analyse discriminante reposant exclusivement sur les variables de la couche ossoïde a permis de classer correctement plus de 84 % des poissons d'après le mode d'alimentation après 8 jours de croissance. Cette technique a été vérifiée sur le terrain à Java (Indonésie) à l'aide de tilapias élevés en cages. Il a ainsi été possible de classer correctement 86 % des poissons en croissance et non en croissance de la population après une période de 21 jours. De telles mesures peuvent permettre de compléter les données recueillies à partir de l'espacement des anneaux de croissance sur la partie calcifiée de l'écaille et ainsi de préciser exactement le taux de croissance récent de chaque poisson.

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Doyle et al. (1987), Matricia et al. (1989), and Talbot and Doyle (1990) demonstrated that the spacing of outer calcified circuli on fish scales (CIRC) was strongly associated with the recent growth rate of that fish, i.e. the growth rate averaged over the previous week to a month. These observations were made on a genus of tropical fish (*Oreochromis* spp., the mouth-brooding tilapias) raised in aquaria as well as in their natural habitats. This relationship has also been shown to be quantifiable in other species (McNaughton 1986; Talbot et al. 1988) and is presumably true of many fish that show annual or seasonal checks in circulus spacing. Possible applications of the CIRC growth estimator to experimental studies, tropical fisheries, and environmental monitoring have been discussed in Doyle et al. (1987) and Talbot and Doyle (1990). The CIRC measurement technique differs from conventional size-at-age estimates derived from temperate fishes in that growth can be measured at any position on the scale, so that it is not dependent on the presence of growth checks, or "annuli." However, there is a situation in which the technique described in Doyle et al. (1987) will not work, namely when

"current growth" is being estimated for fish that are not actually growing. Overestimation of current growth rate may occur because circulus spacing records only the most recent growth rate at the margin of the scale. If a fish stops growing completely during or prior to the experiment so that new circuli are not being formed, CIRC will be calculated for the wrong time interval. The practical objective of the experiment was to improve the accuracy and increase the range of applicability of the CIRC growth estimation technique by identifying such nongrowing fish.

Scales grow by peripheral expansion of the margin, and because fish have a constant number of scales, the rate of scale growth must be proportional to the rate of growth of surface area (Glen and Mathias 1985). Neave (1940) found that the periphery of older scales (the osseoid layer) was not calcified. The osseoid and the zone of calcification together form the zone of growth of the osseous layer (Wallin 1957; "the growing edge of the scale," Dietrich 1953). The osseous layer can be divided into three zones: (1) outermost is the layer of uncalcified osseoid, (2) a zone of calcification, and (3) the entirely calcified part of the scale. After the termination of calcification, the osseous layer is not subject to any further changes except as a result of resorption due to loss of weight (Matlock et al. 1987).

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In preliminary experimentation, we observed that the width of the osseoid layer varied from fish to fish. In nongrowing fish, this osseoid layer quickly disappears, either because it is calcified (Sire and Géraudie 1983) or because it is resorbed (Bilton 1975). We tested the hypothesis that the width of the osseoid layer is related to the rate of growth of the fish over a very short interval using both regression models and discriminant analysis.

Materials and Methods

Experiment I: Comparison of Fed and Unfed Fish

Tilapia (*Oreochromis mossambicus*) from a single cohort were grown separately in 0.5-L containers until approximately 0.5 g in weight and then transferred to separate 5-L containers. Two treatments were imposed on two groups of 25 fish each: (1) ad libitum feeding and (2) starvation. Mean sizes and variances were approximately equal in the two groups at the beginning of the experiment (mean (variance) of length (in millimetres): 36.3 (30.2) versus 35.7 (29.2) in the fed and starved treatments, respectively). The fish were fed tropical fish flakes and pellets. Measurements of standard length (± 0.5 mm) and wet weight (± 0.1 g) were taken at the beginning of the treatments and every 4 d for a period of 20 d. Although the starved fish were not fed, some grazing on the container walls was observed.

Scales were removed each time the fish were measured. On day 4, the first scale taken was the fourth scale behind the opercular bone two rows above the lateral line on both sides of the body (two scales). On subsequent sampling dates, the scale on the same row and immediately behind the scale sampled on the previous date (i.e. fifth scale on day 8, sixth scale on day 12, etc.) was removed. Scales were immediately preserved in 10% buffered formalin to protect the fragile outer margin of the scale. The scale sample thus consisted of two scales per fish per collection date. In the final analysis, 39 fish were used (20 fed, 19 starved) for which there was a continuous time series for the duration of the experiment. In preliminary experiments, we determined that the scales sampled for this experiment did not differ systematically in morphology in accordance with their sequence on the body (Nicholas 1987).

Experiment II: Testing the Technique in a Practical Situation

The scale ossification method for detecting growth was tested with tilapia (of the genus *Oreochromis*) grown in submerged cages in a freshwater reservoir in Central Java, Indonesia. Fish were purchased from two traditional pond farmers in West Java. Farmer A's fish were size graded ("collimated," Doyle and Talbot 1986) to a tolerance of 4 mm into four groups (68–72, 73–76, 77–80, and 81–84 mm). Farmer B's fish were size graded to 1 mm (39–40, 41–42, 43–44, and 45–46 mm). Each group was maintained in a separate cage. All fish were fed daily with pelleted food, with additional grazing of the abundant algae on the nets, and were allowed to interact freely within cages. Growth in length was estimated from the final size of the fish after 21 d and the average initial size per cage. Thus, growth was detectable for fish that grew more than 4 mm and more than 1 mm with farmer A and farmer B's fish, respectively. The classification variable was based on the same size-grading criterion, i.e. any fish whose size was still within the original size-graded tolerance limits was classified as nongrowing. The theoretical implication of collimation to the study of growth rates has been discussed by Doyle and Talbot (1986).

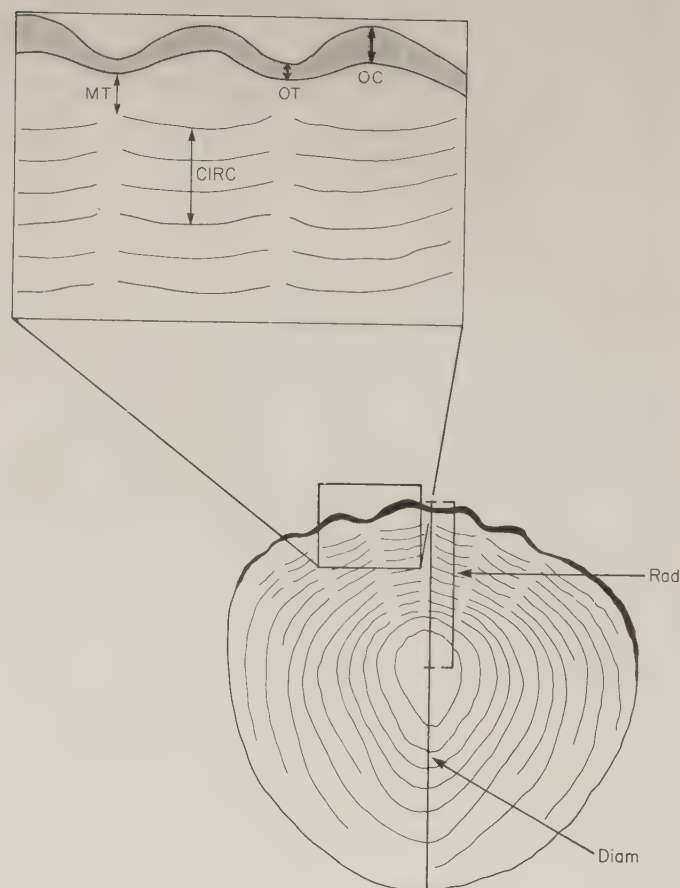


FIG. 1. Representation of a tilapia (*Oreochromis* sp.) scale, showing the measurements taken in this study. Diam = scale diameter, Rad = scale radius; MT = marginal trough, OT = osseoid trough, OC = osseoid crest, CIRC = spacing of first to fourth circulus.

Scale Measurements

Details of the CIRC measurement technique are given in Doyle et al. (1987) and subsequent papers (reviewed in Talbot and Doyle 1990). Scales were wet-mounted on a microscope slide and the spacings of circuli measured at a standard magnification. All measurements (Fig. 1) are derived from the particular structure of ctenoid scales (Sire 1986) of the family Cichlidae, although the basic methodology applies to all scales.

We measured the osseoid layer (Maekawa and Yamada 1972, fig. 1; Wallin 1957, fig. 5) where the most immediate growth-dependent variation occurs. We took several types of measurement for evaluation of the effect of growth on the osseoid layer as follows: (1) the spacing between the first and fourth circulus on the anterior edge of the scale — this is the standard CIRC measurement of earlier studies (Doyle et al. 1987); (2) the osseoid crest (OC) — this is the width of the uncalcified tissue from the outer edge of the scale to the calcification front at the end of a scale radius; (3) the osseoid trough (OT) — this is the width of this same uncalcified tissue at the trough between radii; and (4) the marginal trough (MT) — this is the width from the edge of the calcification front on the scale to the tip of the first circulus at a trough between radii.

The MT measurement includes ossified or ossifying material and is subject to scale resorption. The osseoid measurements are more responsive to short-term changes in the status of the fish than MT. The diameter and radius of the scale were also measured. Regenerated and deformed scales were discarded.

Statistical Analysis

Feeding treatments in the laboratory (experiment 1) were designed to increase the variance in growth rate to a maximum, with a substantial fraction of fish with zero or negative growth. The mean growth rates were expected to be different between treatments, with a significant amount of variation in negative or positive growth overall. However, some fish did not gain weight in the fed treatment and some fish grew slightly in the starved treatment. The relationship between our scale measurements and growth rate is expected to be a continuous bivariate distribution (model II regression)

$$DW_{ij} = \alpha + \beta_1 MT_i + \beta_2 OT_i + \beta_3 OC_i + \epsilon_{ij}$$

where DW_{ij} is the change in weight of individual j at time i and the independent variables as above. The mean scale measurements per sampling time and fish were used in the analysis.

The discriminant analysis model used was the following:

$$\alpha_1 MT + \alpha_2 OT + \alpha_3 OC + \alpha_4 MT^2 + \alpha_5 OT^2 + \alpha_6 OC^2 \\ = c + \beta_1 (\text{FEEDING TREATMENT}) + \epsilon.$$

The dependent variables include second-order polynomial (quadratic) terms. Only measurements of the osseous layer are included in this model, which thus consists of the most current information available. The discriminant analysis we use in this study loses some accuracy, since growth (a continuous variable) causes variation in scale measurement directly, while feeding treatment causes variation in growth rate. We nevertheless base the analysis on the ability of scale variables to discriminate between feeding treatments, which is a conservative test of its ability to identify nongrowing fish. Another possible classification separates growing from nongrowing fish into two groups regardless of treatment. However, a fish classified as nongrowing in one interval would not necessarily be classified in the same category at other intervals. Since the individual fish is the unit of analysis and we examine cumulative growth over several intervals in this paper, using the treatment classification system is more appropriate.

Results

Experiment I

The starved fish on average lost weight, while fed fish gained weight (Fig. 2A). These mean growth rates were significantly different overall and on every individual date except day 12 following a long weekend during which the fish were not fed adequately (Fig. 2B). From this result, we expected the scales of growing fish to continue to grow, while those of starved fish were expected to stop growing or be resorbed. The distance between the first and fourth circuli (CIRC) was not significantly different between treatments after 12 d of growth, as expected if no additional circuli were deposited when growth stopped in the starved group ($F = 0.708$, $df = 1,32$, $p = 0.406$). The correlation between CIRC and growth rate overall (both treatments pooled) at day 12 was close to zero ($r = 0.094$). Within treatments, however, the correlation became 0.65 ($p < 0.01$) for fed fish and -0.39 ($p > 0.05$) for starved fish, despite the lower variance in change in weight for the starved treatment (Fig. 3). This confirmed that in this experiment, CIRC measurements on the calcified portion of the scale were a reliable record of recent or current growth when fish were actually gaining length or weight (Matlock et al. 1987; Doyle et al. 1987; Talbot and Doyle 1990) but were a poor indicator of current growth status when growth was zero or negative.

Since CIRC indicated rate of growth prior to the beginning of the experiment in nongrowing fish, the negative correlation between CIRC and growth in the starved treatment may have indicated that fast-growing fish prior to the beginning of the experiment tended to lose more weight once the starvation treatment began.

The measurements taken on the edge of the scale as measures of resorption (MT) or ossification (OC, OT) were all significantly different between treatments 8 d after the beginning of the experiment (Table 1; Fig. 2C–2E). Furthermore, the OC variable reacted faster to change in growth than the other measured variables (Fig. 2E). The rapidity with which the osseoid reacts to changes in growth rate is further demonstrated by the elimination of the statistical differences between treatments of the osseoid measurements of day 16 following the drop in growth of the fed fish on day 12. Only MT remained (highly) significant during this period (days 12–16), indicating that it responds more slowly than OT or OC, perhaps because it involves ossification. Once established, the treatment effects are more pronounced with MT.

To evaluate the efficiency of osseoid measurements to distinguish between growing and nongrowing individuals in a population, we calculated discriminant scores for each sampling day (4–20, Table 1). The classification success for each discriminant analysis function and date was calculated (Fig. 4). The estimated parameters of the discriminant function for each date were also applied as classification criteria on all other dates. This yielded classification frequencies for days 4–20 based on the five discriminant functions from scale measurements of days 4, 8, 12, 16, and 20.

Several conclusions were derived from these results. Firstly, each discriminant analysis gave optimal results on the sampling date from which it was derived, but more importantly the discriminant functions for days 8–20 gave equivalent classification frequencies on all dates, with the exception of the “day 20” discriminant function applied to day 8 (Fig. 4). This implies that no further increase in resolution can be obtained by using more than 8 d of growth history, or conversely that growth history is accurately reflected in the morphology of the scale within 8 d. The discriminant function estimated from data obtained after 4 d of growth resulted in proper classification of 70% of the fish, but this result was not statistically significant (Table 1). The percentage increased to 84% after 8 d (Table 1). It is therefore possible to discriminate effectively between growing and nongrowing fish after only 1 wk of growth. Since OC is able to react to growth in that short period (Fig. 2E), the inability of the discriminant function to give significant results after 4 d is partly because the function is based on a combination of variables with a range of reaction times.

The MT measurement gives consistently the best differentiation between treatments after 1 wk, followed in order by OT and OC. On day 4, however, OC had the greatest discriminatory power, followed by OT and MT (Table 1). Since the osseoid measures (OC and OT) were not significant on day 16, immediately following day 12 in which the change in weight was not significantly different between treatments, and since MT remained highly significant throughout, we conclude that OC and OT are more responsive to short-term changes (in as little as 4 d in our study), but that MT is more powerful after 1 wk of integration of body growth on scales. This is perhaps because MT includes some ossified or ossifying portion of the scale that is resorbed in starving fish. Ossified tissue is more energetically expensive and metabolically stable and may result

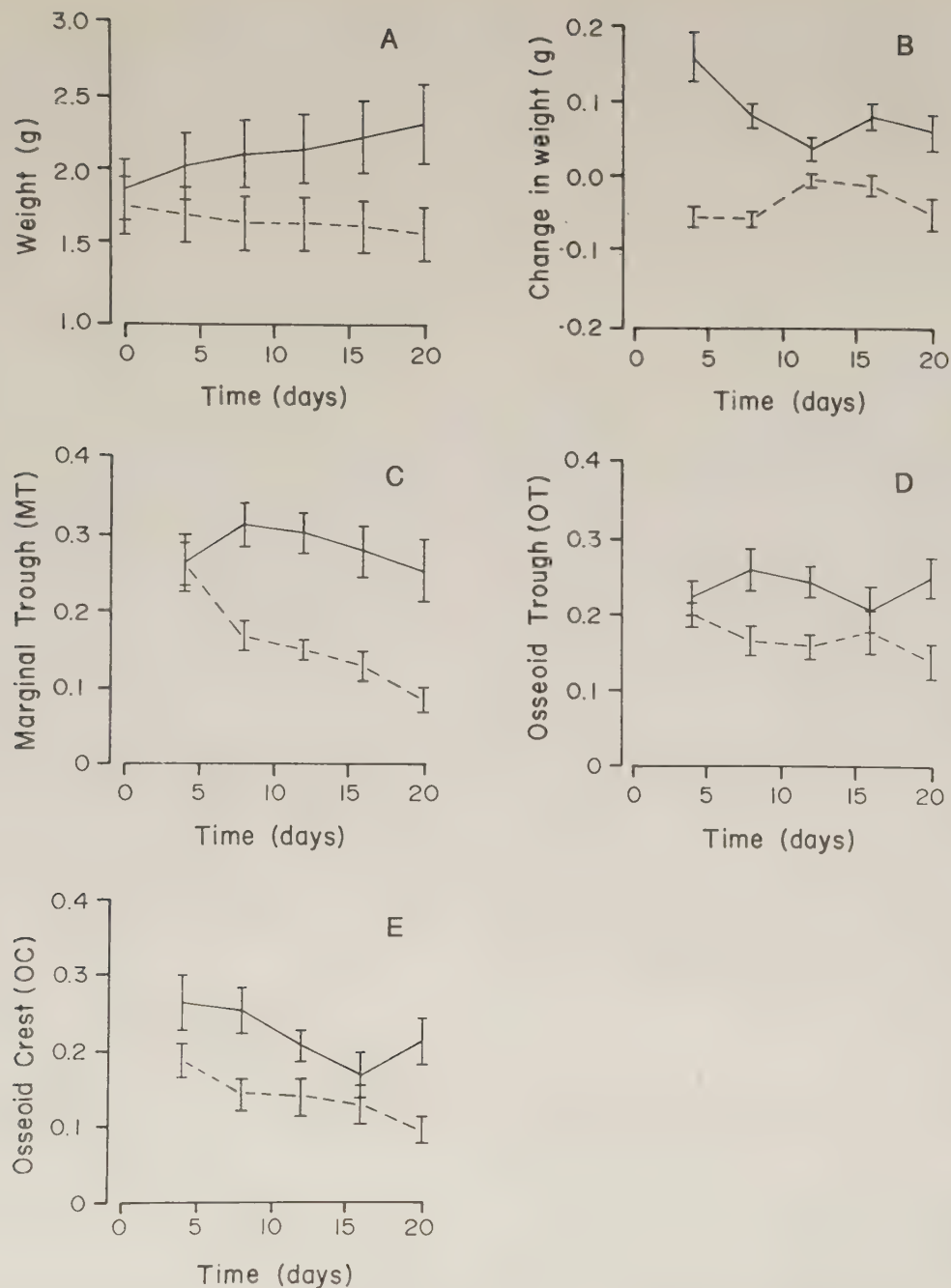


FIG. 2. Observed changes in scale measurement over time. Solid lines represent fed fish; broken lines represent starved fish. Error bars are standard errors. (A) Weight; (B) change in weight (DW); (C) marginal trough (MT); (D) osseoid trough (OT); (E) osseoid crest (OC).

in the formation of a permanent check on the scale after growth resumes.

Experiment II

The particular discriminant function derived in experiment I could not be applied to the Indonesian population because of the extensive hybridization between species and populations of tilapia in Indonesia, making positive identification of a particular species or species complex difficult (Macaranas et al. 1986) and because of the different experimental design. A new discriminant analysis function needed to be derived based on classification as growing and nongrowing fish. Figure 5 shows the bivariate distribution of MT and OT with the observed and predicted classification by discriminant analysis of fish on

farm A. Again, the classification success was very high (85.7%, Table 2). The number of nongrowing fish in farmer B's sample was too small for a proper discriminant analysis (there were no fish which had negative growth rates), but the initial size range was lower and change in length was more accurately measured. In this replicate (farm B), a regression model was judged appropriate. The observed correlation between MT and change in length was 0.89 ($n = 102$, $p < 0.0001$) and that of CIRC and change in length was 0.62. The other scale measurements were not taken for the farm B sample.

If the fish from farm A classified as nongrowing by the discriminant analysis are removed, the correlation between CIRC and change in length changes from 0.759 ($n = 56$, $t = 8.567$,

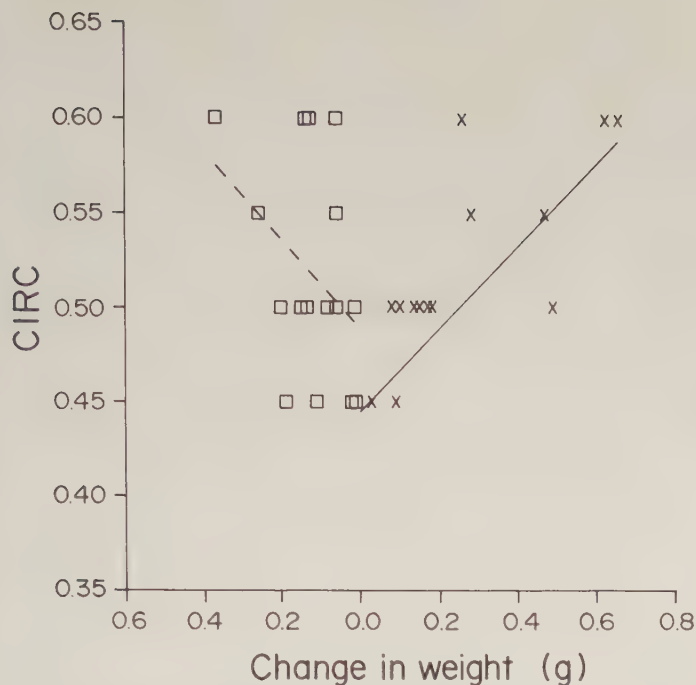


FIG. 3. Relationship between CIRC and DW at day 12 for fed (crosses and solid line) and starved (squares and broken line) fish ($r = 0.65$ for fed fish and $r = -0.39$ for starved fish).

TABLE 1. Standardized discriminant analysis parameters for each of the models tested in experiment I. "Correct" is the percentage of fish correctly classified as fed or starved. See text for further explanation. $^{NS}p > 0.05$; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Dependent variable	Day 4	Day 8	Day 12	Day 16	Day 20
MT	0.005 ^{NS}	0.586***	0.667***	0.587***	0.708***
OT	0.164 ^{NS}	0.429**	0.500**	0.119 ^{NS}	0.606**
OC	0.354 ^{NS}	0.446**	0.332*	0.173 ^{NS}	0.632**
MT ²	0.021 ^{NS}	0.533***	0.604***	0.477**	0.675**
OT ²	0.188 ^{NS}	0.403*	0.464**	0.102 ^{NS}	0.523*
OC ²	0.346 ^{NS}	0.384*	0.188 ^{NS}	0.114 ^{NS}	0.543*
Correct	70.4	84.2	86.1	87.5	84.2

$p < 0.0001$) to 0.794 ($n = 27$, $t = 6.527$, $p < 0.0001$). Within the fish classified as nongrowing, the CIRC/change in length correlation is -0.149 ($n = 29$, $t = 0.784$, $p = 0.44$).

Discussion

Estimates of the width of the "osseoid layer" (Wallin 1957) and of the resorption of the scale (MT, Bilton 1975) are good indicators of the current growth status of fish. We show that the width of the osseoid (1) provides the most up-to-date information on the growth rate of an individual, (2) reacts to changes in growth rates in less than 1 wk, and (3) can be used effectively to discriminate between growing and nongrowing fish in a sample of fish of unknown growth rate.

Several studies on the physiological activity of scale formation provide evidence for variable rates of ossification. Ottaway (1978) demonstrated, using DNA and glycine incorporation analysis, that the fast-growing section of the scale is the anterior margin ("the scale-forming cells") and that the rate of cellular activity was very different for fed and starved fish. It was also evident in Ottaway's study that the cellular activity at

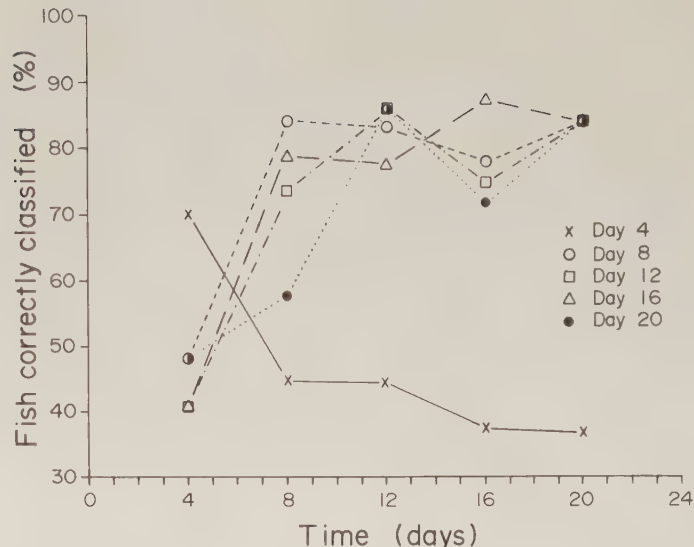


FIG. 4. Changes in the success of the discriminant function classification with time. A function was calculated for each sampling day and applied to the other days. Day 4 classification was not adequate for any data set except day 4, while other dates were similar, except for the slightly reduced classification success for the day 20 function applied on day 8. See text for more detail.

the margin of the scale is most affected by feeding. Adelman (1987) reviewed the literature on uptake of radioactive amino acids as indices of current growth rate in fish. Amino acid uptake demonstrates that growth of scales in fish is sensitive to changes in growth rate of the individual. Furthermore, the rate of calcification plays a role in determining the width of the osseoid layer. Sire and Géraudie (1983) determined that the mineralization of the "osseous" layer occurs very rapidly, so that the width of the "osseoid" layer decreases dramatically in a week if no new "osseoid" tissue is formed. In regenerated scales, Sire (1982) found that calcification was extensive throughout the new scale after only 7 d, supporting the evidence that calcification occurs quickly enough to cause differences in thickness of the osseoid margin in 7 d.

In order for the circulus spacing/growth relationship to hold over an extended period of growth, circuli must be stable on a scale. Sire (1986) demonstrated that the size and orientation of the circuli do not change after they are calcified. Crawford (1988) and Bennett (1988) both provided evidence that the spacing of circuli away from the margin of the scale is correlated with the growth of the fish at the time of scale deposition.

There is some question as to the presence of bias at extremes of growth rates when estimates are derived from the ossified portion of the scale. Although it has been shown that circulus spacing is a good estimator of recent growth in growing fish (the correlations are generally in the order of 0.7–0.8 with instantaneous change in length (Talbot and Doyle 1990)), circulus spacing from very slow- or fast-growing fish may not provide enough information to correctly estimate rate of growth during a given interval. Much of the error in estimating instantaneous growth by circuli spacing stems from the different units of measurement. Growth rate is measured on a temporal basis, i.e. change in length over a 3-wk interval, whereas circulus spacing is measured on a spatial scale. The time over which scale measurements reflect growth is unknown, unless a specific time marker is introduced on the scale (e.g. a fluorescent dye or a check caused by a change in environment, Bennett 1988). Therefore, in very slow- or very fast-growing fish, esti-

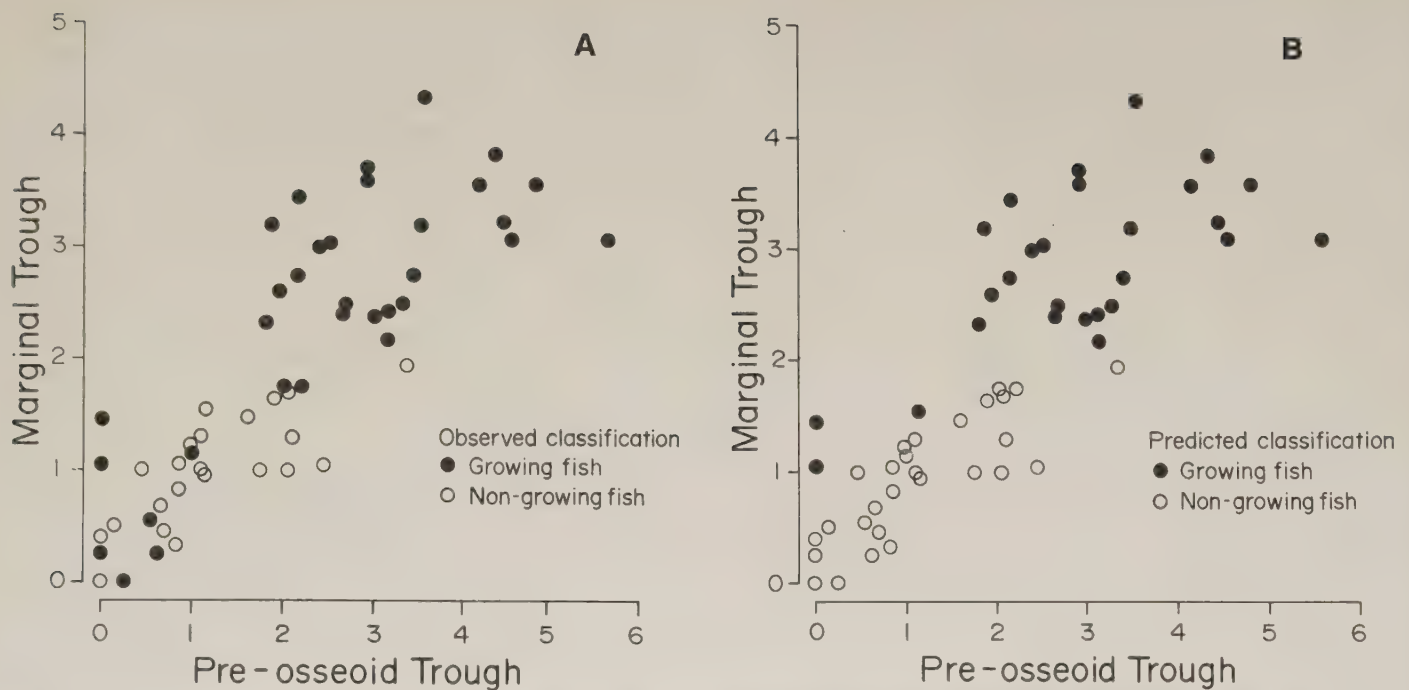


FIG. 5. Bivariate plot of marginal trough (MT) and osseoid trough (OT) showing the predicted and observed classification of fish from farm A in Indonesia, as nongrowers according to the tolerance rule described in the text. Any fish which grew less than 4 mm in length over the experimental period was classified as a nongrower. (A) Observed classification; (B) predicted classification.

TABLE 2. Standardized discriminant analysis parameters for experiment II. "Correct" is the percentage of fish correctly classified as fed or starved. See text for further explanation. * $p < 0.001$.

Dependent variable	Experiment II
MT	0.623*
OT	0.473*
OC	0.570*
MT ²	0.623*
OT ²	0.463*
OC ²	0.497*
Correct	85.7

mates may not be related to the time period under scrutiny. With fish whose current growth rate has stopped, CIRC measurements used alone will overestimate present growth. Procedures such as the ones described in this paper should be employed to detect nongrowth and to delete these individuals from the sample before using CIRC to estimate the growth rates of the remaining fish. The method described in this paper should be used in combination with circulus spacing (Doyle et al. 1987) when monitoring the growth rate of individual fish over a relatively short, current period of time, whenever tagging of fish is impossible or undesirable.

The combination of different types of information on scales, both calcified and uncalcified, could be used to reconstruct the ontogeny of instantaneous growth of untagged fish over an extended period of time where past periods of no growth would be revealed by the presence of "checks" in the circulus pattern in the postmarginal section of the scale. The ability to detect changes in growth rates in a week may be an important contribution to nutrition, disease, or environmental monitoring

studies where short-term effects are concerned. It may also be useful in studies where change in size itself increases the complexity of the analysis and conclusion from data. Multiple predictor variables which include both change in size and size itself invariably suffer from autocorrelation problems. Such a situation is often found in studies in which treatmentwise change in biomass or behavioural feedback loops associated with size hierarchies are observed. In such cases, indicator variables, such as CIRC and ossification growth estimators, glycine uptake by scales (Adelman 1987), or RNA/DNA ratios in tissues (Bulow 1970) may be more useful than change in size itself.

The statistical misclassification of some of the fish by feeding treatment in the laboratory discriminant analysis is partly due to a real overlap in growth rates between the treatments; some fish in the fed treatment did not grow much over the experimental period, while some fish in the starved treatment did not lose weight. This may have introduced a biological rather than a statistical error (i.e. the fish were not misclassified by the discriminant analysis in as much as they were misclassified as a result of their own growth rates). The growth rates of our fish in individual containers were much slower than what would normally be expected from wild populations. We obtained a mean specific growth rate of $11\% \cdot \text{mo}^{-1}$, which is much less than the $20\% \cdot \text{mo}^{-1}$ observed in Indonesia. Since scales of fast-growing fish also grow faster than those of slow-growing fish, we consider our 1-wk response time of scales to changes in growth rates to be a maximum for this species. In general, when growth rates are more variable and the mean growth rate is greater, discrimination time must be significantly shorter than observed in this study.

We have demonstrated that growth information on scales of fish can be useful in many applications. With a very limited amount of technology and resources, a growth trajectory can be reconstructed based entirely on scales. Further research on the

correlated effects of factors other than growth rates on scale formation may be necessary to make this technique unbiased in any application.

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Inferring the Summer Distribution of Migratory Pacific Hake (*Merluccius productus*) from Latitudinal Variation in Mean Lengths-at-Age and Length Frequency Distributions

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Average summer distribution of abundance and biomass for the migratory population of Pacific hake (*Merluccius productus*) is described by age, length, and latitude, based on a model proposing that (1) changes in mean length-at-age with latitude and (2) the nonnormal (skewed to the right) appearance of length-at-age distributions near southwest Vancouver Island could be caused by length-dependent migration velocities and changing migration velocity with latitude. Our model uses mean length-at-age data collected annually near four latitudes from California to Vancouver Island, length frequency data collected annually near southern Vancouver Island from 1978 until 1988, and hydroacoustic data collected triennially from 1977 until 1986. We conclude that (1) hake slow their northward migration from the spawning grounds off southern California as they approach the productive feeding grounds off southwestern Vancouver Island, (2) older (larger) hake migrate further north than younger (smaller) hake, and (3) older hake are more abundant in Canadian waters than younger hake. Although all three conclusions have previously been partly substantiated, we show that seemingly undramatic patterns in the mean, variance, and distribution of lengths-at-age can be remarkably revealing about the distribution of a migrating fish population.

On décrit la distribution estivale moyenne de l'abondance et de la biomasse de la population migratrice de merlu du Pacifique (*Merluccius productus*) en fonction de l'âge, de la longueur et de la latitude en utilisant un modèle suivant lequel 1) les variations des longueurs moyennes par âge en fonction de la latitude et 2) les distributions apparemment non normales (déplacement de la courbe vers la droite) des longueurs par âge près de la côte sud-ouest de l'île de Vancouver pourraient être dues au fait que la vitesse des poissons lors de leur migration dépendrait de leur taille et de la latitude. Les données utilisées dans notre modèle sont les suivantes : longueurs moyennes par âge, mesurées chaque année à quatre latitudes entre la Californie et l'île de Vancouver, fréquence des longueurs établie chaque année près de la partie sud de l'île de Vancouver de 1978 à 1988 et données hydro-acoustiques prises tous les trois ans de 1977 à 1986. Nos conclusions sont les suivantes : 1) le merlu ralentit sa migration vers le nord quand, après avoir quitté ses aires de fraie au large du sud de la Californie, il approche ses riches aires d'alimentation au large de la côte sud-ouest de l'île de Vancouver, 2) les merlus plus âgés (plus gros) migrent plus au nord que les jeunes (plus petits) et 3) les merlus plus âgés sont plus abondants que les jeunes dans les eaux canadiennes. Bien que ces trois conclusions aient déjà été avancées avec à l'appui des preuves partielles, la présente étude montre que la structure des données sur la moyenne, la variance et la distribution des longueurs par âge, même si elle semble banale, peut en dire étonnamment long sur la distribution d'une population migratrice de poissons.

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For the first few years of their life offshore, Pacific hake (*Merluccius productus*) reside year-round within a few hundred kilometres of the coast of southern California or northern Mexico (Bailey et al. 1982; Bailey and Francis 1985). At about 3 yr of age, hake begin an annual feeding migration at depths of 100–250 m (Bailey et al. 1982; Stauffer 1985) to the continental shelf off the Pacific northwest of the United States and southern British Columbia (Bailey and Francis 1985; Beamish and McFarlane 1985) where summertime oceanographic conditions promote a high rate of primary and secondary productivity (Thomson and Ware 1988; Ware and McFarlane 1989; Thomson et al. 1989). Fishable concentrations of hake occur in Canadian waters from about June until October of each year (Beamish 1981; Beamish and McFarlane 1985). In recent years, Pacific hake landings accounted for the largest tonnage of fish caught in Pacific Canadian waters (e.g. 90.5 kt in 1988 (Saunders and Shaw 1989)).

It is well documented that the northern extent of the feeding migration increases with fish age and length (Beamish and McFarlane 1985; Nelson and Dark 1985) and is probably related to fish length (Francis 1983) and optimum swimming velocity (Ware 1978; Weihs and Webb 1983). The northern limit of migration is not known precisely, but recent Canadian hydroacoustic surveys (Barner et al. 1984 and unpubl. data) put the largest summer concentrations of hake in Canadian waters near southern Vancouver Island (Beamish 1981; Beamish et al. 1982; Barner et al. 1984; Shaw et al. 1987, 1989). Furthermore, Canadian and foreign trawl locations (Fig. 1) clearly delineate the shelf waters off southern Vancouver Island as the prime location for summer fishing. Because large females in particular are routinely found north of Vancouver Island, there is a need to define more precisely the distribution and abundance of the oldest and largest fish. Such information will allow assessment of the contribution of these females to the spawning

stock (Beamish 1981) and assist in the establishment of catch allocations for the Canadian and the United States fisheries (Francis and Hollowed 1985; Swartzman et al. 1987).

We observed two prominent features of hake length-at-age data collected from California to British Columbia from 1978 to 1988 which seemed to be related to migration behavior of hake. First, mean lengths-at-age tended to increase with latitude within samples taken about the same time. This feature is unsurprising because for any age-class the extent of northward migration increases with fish length. Second, distributions of length-at-age showed a tendency to be skewed to the right (Fig. 2). Pacific hake lengths-at-age from a nonmigratory population in the Strait of Georgia (McFarlane and Beamish 1985) appear approximately normally distributed (Welch and McFarlane 1990 and unpubl. data), suggesting to us that the skewness might result from migration. Specifically, if the larger and faster migrating hake of any age-class slowed or ended their migration in Canadian waters, then an accumulation of the larger individuals in Canadian waters could yield perceptibly skewed distributions.

In this paper we use these data, in conjunction with estimates of the summer percentage of abundance-at-age in Canadian waters from hydroacoustic survey data (Dorn and Methot 1990), within a model framework which provided a description of the distribution of abundance of Pacific hake by length and latitude. The model we developed is based on the theory that the swimming velocity of a fish increases as a power function of its length (Ware 1978; Weihs and Webb 1983) and the specific knowledge that Pacific hake segregate by length as they migrate and probably slow their migration as they reach Canadian waters. In this study we elaborate on our suggestion (Smith

et al. 1990) that offshore Pacific hake length-at-age statistics estimated from *samples* at particular latitudes are not representative of *population* length-at-age statistics. Here, we deal with this distinction explicitly by using statistical information from length-at-age samples to estimate the mean, variance, and distribution of the Pacific hake population within the framework of the migration model. In this paper we develop the mathematics of our model, apply it to our data, and appraise the results in the context of understanding migratory hake population dynamics.

As recently indicated by Quinn et al. (1990), the small number of papers on this topic is partly due to extensive data requirements and the need for a complex model structure for studying migration dynamics. In recognition of this, our analysis proceeds with assumptions and simplifications which allow an illustrative assessment of average hake distribution by length and latitude, but which limit its utility in annual management because contemporary conditions, not average conditions, must be the basis for annual management. We wish mainly to demonstrate that subtle and undramatic changes in population statistics (i.e. degree of skewness, mean lengths-at-age, etc.), which might not appear immediately informative of population processes or status, can be quite informative when analyzed within a suitable model framework.

Migration Model

The Data

Pacific hake samples have been collected for the Canadian research program along the Canadian and northern United States

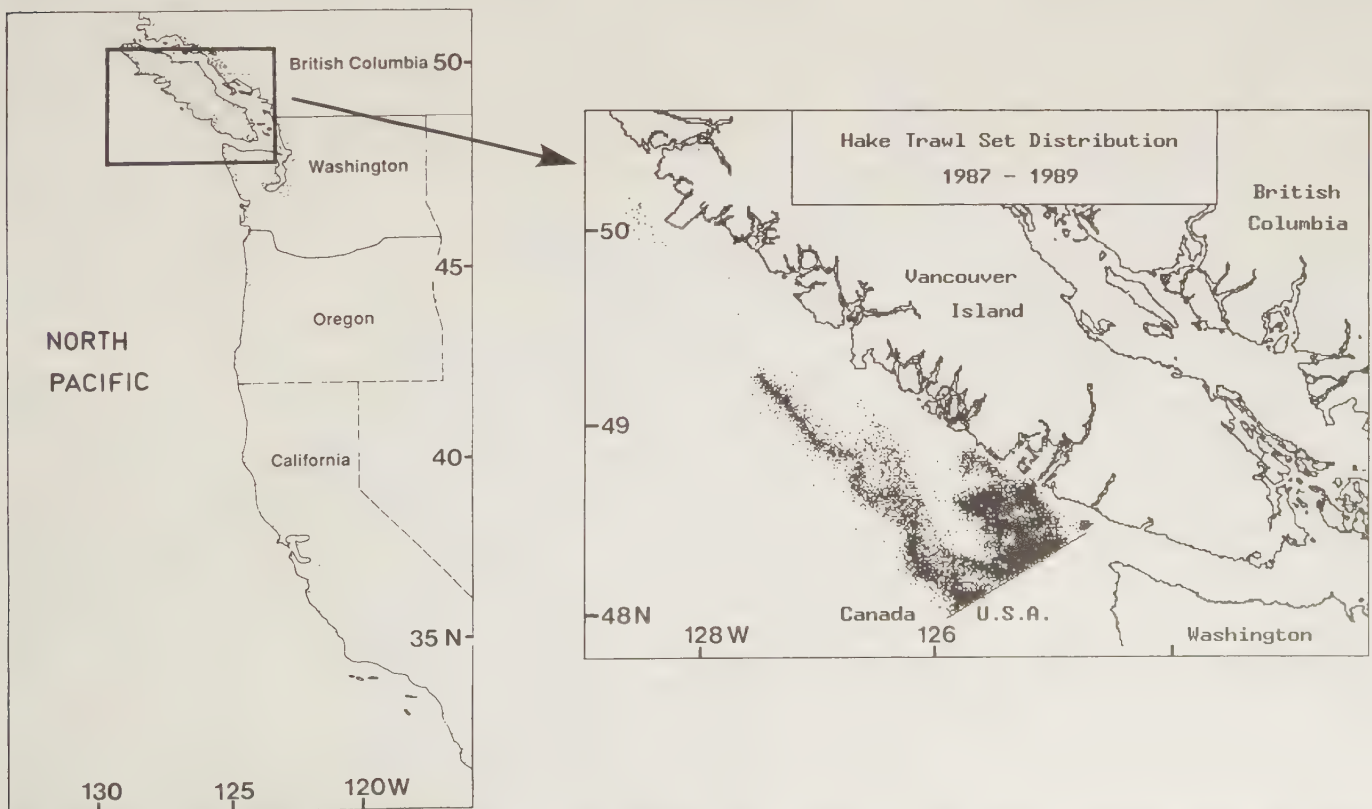


FIG. 1. Western coastal North America with emphasis on the southwest coast of Vancouver Island where the Canadian domestic and foreign national fishing fleets concentrated their efforts during 1987 until 1989. The cluster of trawl sets (dots) adheres closely to the 200-m depth contour where hake tend to concentrate.

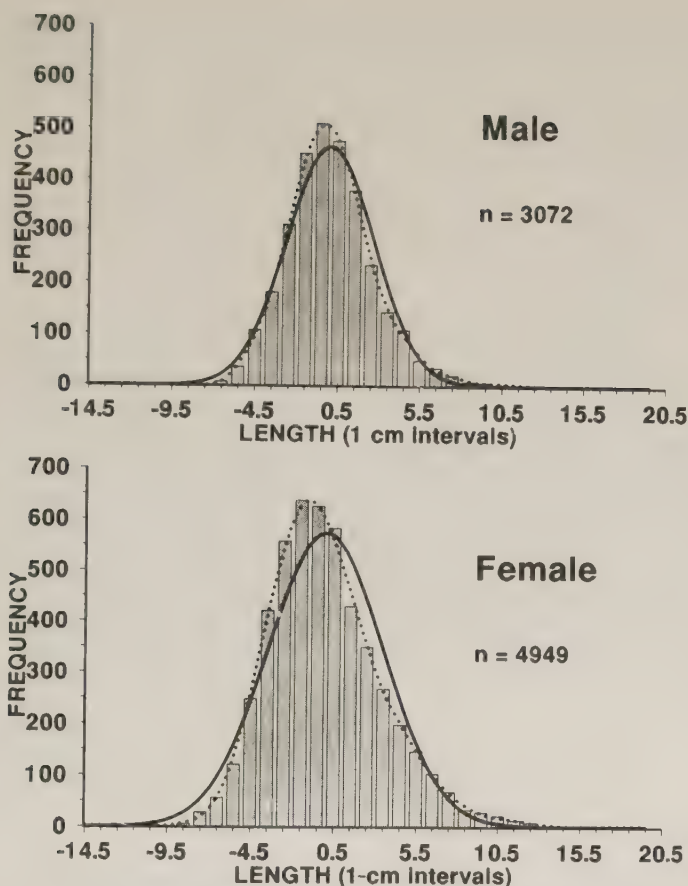


FIG. 2. Frequency distributions for all aged male and female Pacific hake sampled off southern Vancouver Island from 1978 until 1988. Length-at-age samples for each year and age were standardized to a mean of zero and then combined to facilitate recognition of the general feature of right-handed skewness in the distributions. The solid line allows comparison of the sample distribution with a normal distribution fitted to these data, while the dotted line depicts, for example, a skewed distribution.

west coasts by midwater trawl since 1976 (Beamish 1981; Beamish et al. 1982; Barner et al. 1984; Davenport 1985; Shaw et al. 1985, 1987, 1989). Random samples collected onboard research and commercial vessels were sexed and measured for fork length to the nearest millimetre. Otoliths were collected from every fish in a sample and all fish were aged according to the procedures of Beamish (1979). This procedure yielded length-at-age distributions and their corresponding statistics directly. In this paper we analyze the mean, variance, and distribution of lengths-at-age from samples collected by the Canadian research program off southern Vancouver Island near 48.7°N from 1978 until 1988.

Samples for the United States research program have been collected using research and commercial vessels (French et al. 1981; Nelson and Dark 1985) along the United States, and sometimes Canadian, west coast since 1965 by midwater and bottom trawl (Bailey and Francis 1985). However, unlike the Canadian program, the United States program employs a two-stage sampling procedure in which the samples of each sex were first measured for fork length then subsequently only subsampled by length strata to generate age-at-length keys (Kimura 1977; Kimura and Chikuni 1987). Length-at-age distribution statistics can be generated from age-length keys, but at this time only mean lengths-at-age, as determined using the delta method (Kimura 1989), were available for analysis.

Mean length-at-age data from the United States research program were categorized by sex, year (1978–88), season (spring, summer, autumn), and latitude (≈ 41.5 , ≈ 44.5 , and $\approx 47^\circ\text{N}$). We compressed these data by averaging the mean lengths-at-age over the seasons and the years 1978–88 because in this analysis we were interested only in a general assessment of hake distribution by latitude. This averaging of both the United States and Canadian mean lengths-at-age over the years 1978–88 therefore disguised both a general decline over time in mean lengths-at-age, probably due to fishing (Smith et al. 1990), and a marked interannual variability in length-at-age linked to coastal current strength (Smith et al. 1990). By averaging over season we assumed that the average mean lengths-at-age are representative of the mean lengths-at-age of Pacific hake once the population has segregated by length after its feeding migration. To match the United States data, we categorized the Canadian length-at-age data, collected in summer near 48.7°N, by sex alone.

We also summarized the Canadian length-at-age distributional data by combining these data over the years 1978–88 after first scaling each distribution to a mean of zero. In processing the data this way we assume that the form of the distributions does not vary appreciably with mean length-at-age. Scaling the distribution means to zero allowed us to analyze the form of the length-at-age distributions with statistical independence from shifts in mean lengths-at-age.

Triennial hydroacoustic surveys for offshore hake conducted by scientists from the United States since 1977 provided estimates of the summer percentage of abundance-at-age in Canada (Dorn and Methot 1990). These data were summarized to provide an average summer percentage of abundance-at-age (C_j) in Canada over the 11-yr period from 1978 until 1988. We arrived at these average percentages by assigning equal weight to the 1977, 1980, and 1986 surveys over the 10 yr excluding 1983 for a total weighting of 10/11ths for these surveys. El Niño conditions resulted in an unusually large percentage of hake of all age-classes occurring in Canada during the summer of 1983. Thus the 1983 survey results were atypical and we assigned a weight of 1/11th to the 1983 survey.

We used data only for ages 3 and older because younger hake do not undergo a significant annual migration, and they are not well sampled along the west coast by either the Canadian or United States research programs. Hake 14 yr and older showed no trends in length-at-age statistics so, as in Smith et al. (1990), the analysis presented herein combines all hake 14 yr and older into a 14⁺ age-class with a larger sample size. In summary, our data set consists of 48 male and female sample mean lengths-at-age (O_{jd}) for ages $j = 3, \dots, 14^+$ and latitudes $D = 41.5, 44.5, 47$, and 48.7°N and 12 male and female length-at-age distributions (i.e. the distribution of Q_{jd} 's (see Table 1)) for ages $j = 3, \dots, 14^+$ and latitude $D = 48.7^\circ\text{N}$. The subscript i denotes a 1-cm length interval with the midpoint i (centimetres). Our samples are presumed to have been collected at a single point in time (T); therefore we chose $T = 1^\dagger$, where † is an arbitrary unit of measurement. Definitions for all symbols used in our model appear in Table 1.

Model Development

Our primary goal was an examination of the average distribution of abundance of migratory Pacific hake by length and latitude under a particular model structure and set of simplifying assumptions, the most significant being the use of length-at-age data summarized over seasons and the years 1978–88. Conceptually, our model is three-dimensional with the density

TABLE 1. Definitions and units for symbols used to represent model variables and parameters.

Symbol	Units	Definition
T	$\dagger$	Time, measured in the arbitrary unit $\dagger$
D	$^{\circ}\text{L}$	Geographical location (in degrees of latitude)
D_{sL}	$^{\circ}\text{L}$	Theoretical starting latitude, at $T = 0$, when fish of length L are unsegregated by latitude
D_{eL}	$^{\circ}\text{L}$	Theoretical ending latitude, at $T = \infty$, attained by migration of a fish of length L
V_{*L}	$^{\circ}\text{L} \cdot \dagger^{-1}$	Migration velocity at $D = D_{*L}$ for a fish of length L
V_{DL}	$^{\circ}\text{L} \cdot \dagger^{-1}$	Migration velocity at D for a fish of length L
a	$^{\circ}\text{L} \cdot \dagger^{-1}$	Shape parameter introduced in Eq. 4
b	$^{\circ}\text{L} \cdot \dagger^{-1}$	Shape parameter introduced in Eq. 4
r_L	$^{\circ}\text{L}^{-1}$	Instantaneous rate of decline in V_{DL} with latitude for a fish of length L
ϕ	$^{\circ}\text{L} \cdot \dagger^{-1} \cdot \text{cm}^{-\gamma}$	Scaling parameter relating V_{*L} to L
L	cm	Fork length
γ	Dimensionless	Exponential parameter relating V_{*L} to L
l_j	cm	Mean population length-at-age j
K_l	yr^{-1}	Annual rate of change in l_j
ψ_j^2	cm^2	Variance of population length-at-age j
K_ψ	yr^{-1}	Annual rate of change in ψ_j
ξ_L	$\dagger \cdot ^{\circ}\text{L}^{-1}$	Lognormal probability density function (pdf) of V_{*L}
v_{*L}	Dimensionless	Expected value of $\ln[V_{*L}]$ given ξ_L
λ_j	Dimensionless	Variance of $\ln[V_{*L}]$ given ξ_L and age j
K_λ	yr^{-1}	Annual rate of change in λ_j
Ω_j	Dimensionless	Normal pdf of L for the population of fish of age j
X_L	Dimensionless	Transformed lognormal pdf of D for fish of length L
η	$^{\circ}\text{L}$	Mean of X
δ^2	$^{\circ}\text{L}^2$	Variance of X
Γ_j	Dimensionless	Bivariate pdf of L and D for a fish of age j
Γ_{jD}	Dimensionless	Cross-sectional pdf of L for a fish of age j sampled at latitude D
α	cm	Lower bound of the interval $[L = \alpha, L = \beta]$ within which a solution to Γ_{jD} exists
β	cm	Upper bound of the interval $[L = \alpha, L = \beta]$ within which a solution to Γ_{jD} exists
Q_{ijD}	cm^{-1}	Observed frequency of hake sampled at latitude D and of age j occurring within length interval i
f_{ijD}	Dimensionless	Proportion of hake in length interval i of pdf Γ_{jD}
P_{ijD}	cm^{-1}	Predicted frequency of hake sampled at latitude D and of age j occurring within length interval i
i	cm	Index for 1-cm length intervals $i = 1, \dots, 100$
j	yr	Index for ages $j = 3, \dots, 14^>$
O_{jD}	cm	Observed mean lengths-at-ages j for fish sampled at latitude D
ω_{jD}	cm	Predicted mean lengths-at-ages j for fish sampled at latitude D
ρ_{jD}^2	cm^2	Variance of lengths-at-ages j for fish sampled at latitude D
C_j	%	Hydroacoustically measured percent abundance-at-age in Canadian waters
ζ_j	%	Predicted percent abundance-at-age in Canadian waters
W_L	g	Weight for a hake of length L
Θ_1	Dimensionless	Normal negative log-likelihood function for mean lengths-at-age
Θ_2	Dimensionless	Multinomial negative log-likelihood function for length-at-age frequencies
Θ_3	Dimensionless	Normal negative log-likelihood function for percentage abundance-at-age in Canadian waters
$\Theta_{\min}$	Dimensionless	Minimum negative log-likelihood
σ_ω^2	cm^2	Residual variance for the likelihood function Θ_1
N_ω	Dimensionless	Frequency of observed mean lengths-at-age
σ_ζ^2	% ²	Residual variance for the likelihood function Θ_3
N_ζ	Dimensionless	Frequency of measured abundance-at-age in Canada
n_j	Dimensionless	Cumulative frequency of length-at-age j distributions
$\tau_1 - \tau_{10}$	—	Intermediate terms for Eq. 9 (τ_1, τ_2) and several equations in Appendices A and B
F	—	Simplified representation of Eq. 9
M	yr^{-1}	Annual instantaneous natural mortality

distribution of numbers (vertical axis) being related to two horizontal axes of length and latitude for 3- to 14[>]-yr-old hake. Such a bivariate distribution arises through statistical variation in (1) lengths-at-age and (2) the latitude achieved by migrating

fish of the same length. Consequently a fundamental requirement of our model is two stochastic functional forms relating change in the density of numbers-at-age to each of length and latitude.

We start by assuming that the particular migration velocity for a fish of length L (V_{*L}) at the particular location $D = D_{*L}$ is related to fish length L by the power function

$$(1) \quad V_{*L} = \phi L^{0.43} \xi_L$$

(Ware 1978; Weihs and Webb 1983) with scaling and power parameters ϕ and $\gamma = 0.43$, respectively. Ware (1978) and Weihs and Webb (1983) independently derived the value $\gamma = 0.43$ based on theoretical thermodynamic and hydrodynamic arguments, respectively. We also admit lognormal variation in V_{*L} :

$$(2) \quad \xi_L = \frac{1}{\sqrt{2\pi} \lambda V_{*L}} e^{-\frac{(\ln[V_{*L}] - \nu_{*L})^2}{2\lambda^2}}$$

where ν_{*L} and λ are parameters of the lognormal distribution ξ_L and

$$(3) \quad \int_{V_{*L}=0}^{V_{*L}=\infty} \xi_L dV_{*L} = 1.$$

The choice of a lognormal distribution follows from the expectation of multiplicative variation of the random variable V_{*L} for a function of the form of Eq. 1 (Walters 1986).

We permit variation in the migration velocity (V_{DL}) with location D . A functional form which provides for change in velocity V_{DL} or (dD/dT) with location is

$$(4) \quad \frac{dD}{dT} = V_{DL} = \frac{e^{-r_L(D-D_{*L})} (V_{*L} + a)^2 - a^2}{e^{-r_L(D-D_{*L})} (V_{*L} + 2a - b) + b}$$

where T is time, V_{DL} is migration velocity at location D , r_L is the instantaneous rate of change in velocity with location D , and a and b are shape parameters ≥ 0 . This formulation has the desirable properties that

$$(5) \quad V_{DL} = V_{*L} \text{ when } D = D_{*L}$$

$$(6) \quad V_{DL} = 0 \text{ when } D = D_{eL} = \frac{-2\ln\left[\frac{a}{V_{*L} + a}\right]}{r_L} + D_{*L}$$

$$(7) \quad V_{DL} \approx V_{*L} \text{ when } D = -\infty, a \ll V_{*L}, b \ll V_{*L}$$

as well as the asymptotic property that

$$(8) \quad V_{DL} = -\frac{a^2}{b} \text{ when } D = \infty.$$

If $b - 2a > V_{*L}$, then Eq. 4 has the undesirable property of a singularity in the relationship between V_{DL} and D .

When Eq. 4 is integrated over D and T and set to the initial condition of $D = D_{sL}$ at $T = 0$, then

$$(9) \quad \left\{ \frac{br_L(D - D_{sL})(V_{*L} + a)^2 + \tau_1\tau_2}{r_L a^2 (V_{*L} + a)^2} \right\} + T = 0$$

where

$$(10) \quad \tau_1 = 2a^3 + a^2 V_{*L} + 2abV_{*L} + bV_{*L}^2$$

and

$$(11) \quad \tau_2 = \ln[e^{-r_L(D - D_{*L})}(V_{*L} + a)^2 - a^2] - \ln[e^{-r_L(D_{sL} - D_{*L})}(V_{*L} + a)^2 - a^2].$$

Equation 9 must be solved numerically for D , the location achieved after migrating for time T , given values for a , b , D_{sL} ,

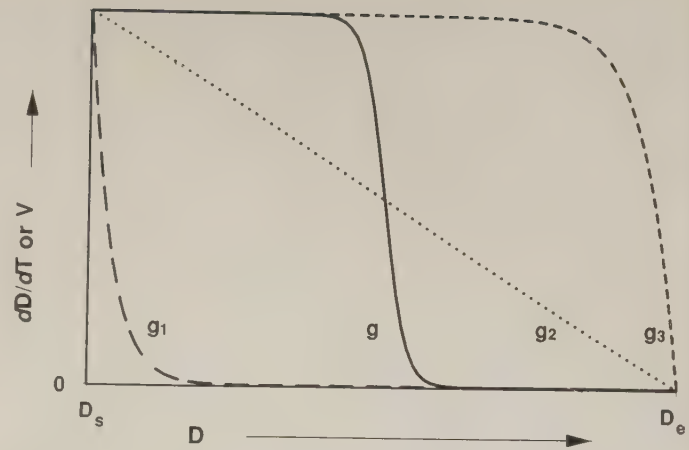


FIG. 3. Representative examples of Eq. 4 which relate migration velocity (dD/dT or V) to a geographical measure of location (D). Examples g and g_1 – g_3 illustrate the form of Eq. 4 for different values of the shape parameters a and b as listed in Table 2.

TABLE 2. Parameter values for the example relationships (Eq. 4) presented in Fig. 3–6. In all examples, $D_s = 0$ and $D_e = 0$.

	Example	a	b	V_{*L}	D_{eL}	λ	T
Fig. 3	g	10^{-10}	10^{-10}	100	100	—	—
	g_1	10^{-4}	10^{+2}	100	100	—	—
	g_2	10^{+2}	10^{+2}	100	100	—	—
	g_3	10^{-2}	10^{-7}	100	100	—	—
Fig. 4a	$T = 1$	10^{-4}	10^{-4}	25	200	0.2	1
	$T = 3$	10^{-4}	10^{-4}	25	200	0.2	3
	$T = 10$	10^{-4}	10^{-4}	25	200	0.2	10
	$T = 100$	10^{-4}	10^{-4}	25	200	0.2	100
Fig. 4b	$T = 1$	10^{-1}	10^{-5}	25	200	0.2	1
	$T = 3$	10^{-1}	10^{-5}	25	200	0.2	3
	$T = 6$	10^{-1}	10^{-5}	25	200	0.2	6
	$T = 7$	10^{-1}	10^{-5}	25	200	0.2	7
Fig. 5		10^{-4}	10^{-4}	25	200	0.2	0–40

and r_L . We obtained solutions to Eq. 9 using the “bracketing and bisection” method (Press et al. 1986).

One important feature of Eq. 4 and Eq. 9 is that they include the variable D_{sL} and define the variable D_{eL} which represent the theoretical starting (at $T = 0$) and ending (at $T = \infty$) locations, respectively, of migration, at which points fish of the same length are unsegregated by location. Note that in the limit as $a \rightarrow 0$, $D_{eL} \rightarrow \infty$. Alternative representations of Eq. 9 for the limiting conditions as the parameters a , $b \rightarrow 0$ are given in Appendix A. Another important feature of Eq. 4 and Eq. 9 is that they provide flexibility in the form of the relationship between V_{DL} and D , as illustrated by the examples in Fig. 3. In Fig. 3, examples g_1 to g_3 are line segments of the generic example g obtained from Eq. 4. They represent different values of the parameters a and b scaled within the horizontal axis boundaries D_{sL} and D_{eL} (Table 2).

Equation 9 is deterministic and does not admit variation in D among fish of length L that have migrated for the same time T for fixed values of a , b , D_{sL} , and r_L . We have already introduced lognormal variation in migration velocity, V_{*L} , at $D = D_{*L}$ (Eq. 1 and Eq. 9); therefore, variation in D can be determined by transforming the lognormal distribution of the ran-

dom variable V_{*L} to a distribution of the (now) random variable D according to

$$(12) \quad X_L = \frac{1}{\sqrt{2\pi} \lambda V_{*L}} e^{-\frac{(\ln[V_{*L}] - v_{*L})^2}{2\lambda^2}} \left| \frac{dV_{*L}}{dD} \right|$$

(Walpole and Myers 1972) where X_L is the probability density function (pdf) for D such that

$$(13) \quad \int_{D=D_{sL}}^{D=D_{eL}} X_L dD = 1.$$

In Eq. 12, V_{*L} is a solution to Eq. 9 given T and D , subject to the constraint $D_{sL} \leq D < D_{eL}$ which ensures that X_L exists for all $T \geq 0$. The derivative dV_{*L}/dD for $a, b > 0$ and the limiting conditions $a, b \rightarrow 0$ is given in Appendix B.

To accommodate different values for D_{sL} and r_L for fish of different lengths, both variables were related linearly to length using

$$(14) \quad D_{sL} = D_{s,0} + 0.01 L(D_{s,100} - D_{s,0})$$

and

$$(15) \quad r_L = r_0 + 0.01 L(r_{100} - r_0)$$

where the parameters $D_{s,0}$, r_0 and $D_{s,100}$, r_{100} are values for fish 0 and 100 cm in length (L), respectively.

Figure 4 demonstrates typical location distributions for two contrasting patterns of migration velocity (V) versus location (D). In the first example (Fig. 4a), migration velocity slows to near zero well before $D \rightarrow D_e$ such that only as $T \rightarrow \infty$ will the population attain D_e . Alternatively, migration velocity decreases rapidly near D_e (Fig. 4b).

The typical pattern in both examples is an increase in the mean of D (η),

$$(16) \quad \eta = \int_{D=D_s}^{D=D_e} DX dD,$$

and in the variance of D (δ),

$$(17) \quad \delta^2 = \int_{D=D_s}^{D=D_e} D^2 X dD - \eta^2,$$

with time T until $V \rightarrow 0$ at which point the variance of D starts to decrease as the faster migrating individuals in the population

slow down (Fig. 5). Note that the integrals in Eq. 16 and Eq. 17 do not have an analytical solution and so they, and those presented later, were calculated numerically using the Runge-Kutta method (Boyce and DiPrima 1977).

To this point we have described a process to model the distribution of fish by the first dimension, location, and in this specific analysis, latitude. Latitude is a consistent measure of distance along a meridian, and hake migration approximates a north-south orientation (Fig. 1).

Our description of the distribution of individuals along the second dimension (length) was subscripted by age. Mean lengths-at-age $j = 3, \dots, 14$ (l_j) were related to age using the reparameterized von Bertalanffy function

$$(18) \quad l_j = l_3 + (l_{14} - l_3) \frac{(1 - K_l^{j-3})}{(1 - K_l^{14-3})}$$

(Schnute and Fournier 1980; Schnute 1981) where K_l is the annual rate of change in mean length-at-age and l_3 and l_{14} are explicitly estimated parameters. Variance in lengths-at-age was related to age using

$$(19) \quad \psi_j = \psi_3 + (\psi_{14} - \psi_3) \frac{(1 - K_\psi^{j-3})}{(1 - K_\psi^{14-3})}$$

(Schnute and Fournier 1980) where ψ_j is the standard deviation of the length-at-age j distribution, K_ψ is the annual rate of change in the standard deviations of lengths-at-age, and ψ_3 and ψ_{14} are explicitly estimated parameters.

A fundamental assumption of our model is that the distribution of lengths-at-age j (Ω_j) for the population of offshore Pacific hake is normal, that is

$$(20) \quad \Omega_j = \frac{1}{\sqrt{2\pi} \psi_j} e^{-\frac{(L-l_j)^2}{2\psi_j^2}}$$

and

$$(21) \quad \int_{L=-\infty}^{L=\infty} \Omega_j dL = 1.$$

We base this assumption on the observation that lengths-at-age for nonmigratory Pacific hake in the Strait of Georgia, British Columbia (McFarlane and Beamish 1985; Welch and McFarlane 1990), are not consistently nonnormal. Furthermore, a normal distribution of lengths-at-age is typical for many

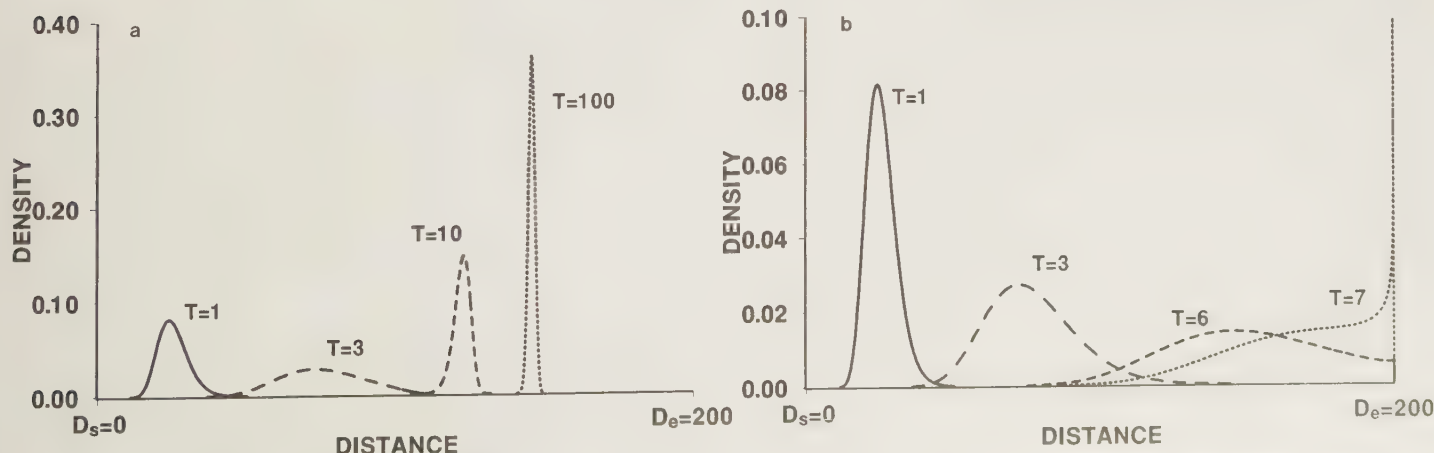


FIG. 4. Typical location (D) distribution patterns over time (T) for a population which slows down (a) gradually (e.g. like g and g_1 of Fig. 3) and (b) suddenly (e.g. like g_3 of Fig. 3) as it approaches the theoretical end point of its migration (D_e). All members of the population would attain D_e at $T = \infty$. Parameter values for these examples are listed in Table 2.

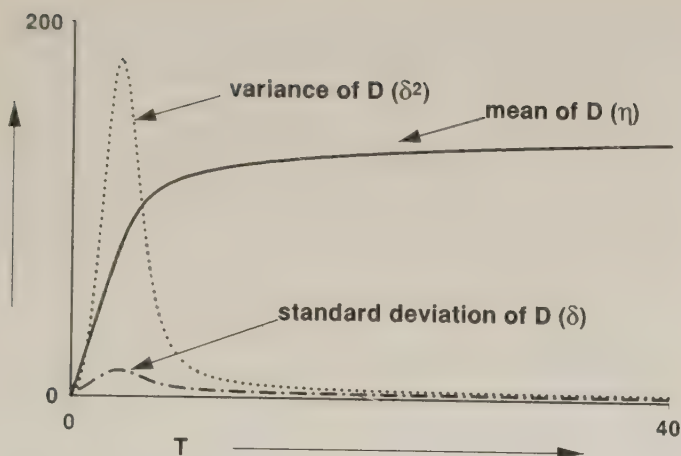


FIG. 5. General trends in the mean of $D(\eta)$, the variance of $D(\delta^2)$, and the standard deviation of $D(\delta)$ over time (T) for a migrating population. Typically, the mean, variance, and standard deviation increase with time (T) until $D \rightarrow D_e$ as $T \rightarrow \infty$. As faster moving individuals within the population approach D_e , slower individuals catch up and compress the variance of D . For this example, Eq. 4 would appear as g in Fig. 3 but with the parameter values listed in Table 2 for Fig. 5.

species of fish (Ricker 1969, 1979). Formal statistical tests (Snedecor 1946) indicated that length-at-age distributions for both male and female Strait of Georgia hake aged 3–14[>] displayed no consistent or significant skewness ($p > 0.5$ for males; $p > 0.3$ for females). In contrast, these same age-classes for migratory hake consistently displayed significant right-handed skewness ($p < 0.01$ for males; $p < 0.001$ for females).

Earlier we stated the premise that migration segregates hake over latitude differentially with respect to length. Thus, sample length-at-age distributions and their associated statistics, collected at any single latitude (D), will not represent population length-at-age distributions or their statistics. However, length-at-age samples collected along the two dimensions of latitude (D) and length (L) have the potential to provide sufficient information to infer population length-at-age distributions and statistics. Our interest therefore becomes the joint distribution of the abundance of fish of age j (Γ_j) by latitude (D) and length (L):

$$(22) \quad \Gamma_j = X\Omega_j = \frac{1}{2\pi\lambda_j\psi_j V_{*L}} e^{-\left\{\frac{(\ln[V_{*L}] - v_{*L})^2}{2\lambda_j^2} + \frac{(L - l)^2}{2\psi_j^2}\right\}} \left| \frac{dV_{*L}}{dD} \right|$$

with

$$(23) \quad \int_{D=D_{sL}}^{D=D_{eL}} \int_{L=\alpha}^{L=\beta} \Gamma_j dL dD = 1.$$

As for Eq. 12, V_{*L} in Eq. 22 is a solution to Eq. 9 given T and D , with $D_{sL} \leq D < D_{eL}$. The interval $[L = \alpha, L = \beta]$ in Eq. 23 defines the continuous segment within the interval $[L = -\infty, L = \infty]$ where $D_{sL} \leq D < D_{eL}$, and thus a solution to Γ_j exists for all $T \geq 0$.

Note that in Eq. 22 we subscripted the parameter λ by age j to account for interage variability in the latitudinal distribution of hake of the same length. We used the functional form

$$(24) \quad \lambda_j = \lambda_3 + (\lambda_{14} - \lambda_3) \frac{(1 - K_\lambda^{j-3})}{(1 - K_\lambda^{14-3})}$$

to relate the λ_j 's to age where λ_j is the standard deviation of $\ln[V_{*L}]$ (from Eq. 2) for a fish of age j , K_λ is the annual rate of change λ_j , and λ_3 and λ_{14} are explicitly estimated parameters.

Length-at-age distributions sampled at particular latitudes D thus represent cross-sections of lengths through the joint distribution of latitude and length. The distribution for such a cross-section is represented symbolically by Γ_{jD} (e.g. the thick solid lines in Fig. 12 (for ages 3 and 14[>]). Almost certainly

$$(25) \quad \int_{L=\alpha}^{L=\beta} \Gamma_{jD} dL \neq 1,$$

and thus the mean (ω_{jD}) and variance (ρ_{jD}^2) of Γ_{jD} are determined using

$$(26) \quad \omega_{jD} = \frac{\int_{L=\alpha}^{L=\beta} L \Gamma_{jD} dL}{\int_{L=\alpha}^{L=\beta} \Gamma_{jD} dL}$$

and

$$(27) \quad \rho_{jD}^2 = \left(\frac{\int_{L=\alpha}^{L=\beta} L^2 \Gamma_{jD} dL}{\int_{L=\alpha}^{L=\beta} \Gamma_{jD} dL} \right) - \omega_{jD}^2,$$

respectively (Walpole and Myers 1972).

To model our length frequency data, we calculated the proportion (f_{ijD}) of the cross-sectional distribution (Γ_{jD}) at latitude D for a fish of age j occurring within each 1-cm length interval i :

$$(28) \quad f_{ijD} = \frac{\int_{L=i-0.5-\omega_{jD}}^{L=i+0.5-\omega_{jD}} \Gamma'_{jD} dL}{\int_{L=\alpha}^{L=\beta} \Gamma_{jD} dL}$$

where i is an integer between α and β , and all elements of every interval $[i - 0.5, i + 0.5]$ fall between α and β . The distribution Γ'_{jD} represents Γ_{jD} scaled to a mean of zero to be consistent with the standardized mean lengths-at-age of our samples. The predicted number of fish (P_{ijD}) within an interval i at latitude D for at length-at-age j distribution with sample size n_j is therefore

$$(29) \quad P_{ijD} = n_j f_{ijD}$$

and

$$(30) \quad n_j = \sum_{i,j,D} Q_{ijD}$$

where each Q_{ijD} is the observed frequency of hake sampled at latitude D and of age j occurring within length interval i .

The summer percentage of abundance-at-age of hake in Canadian waters (ξ_j), i.e. north of the Canada-U.S. border near 48.3°N, was calculated using the following particular application of Eq. 23:

$$(31) \quad \xi_j = 100 \int_{D=48.3}^{D=D_{eL}} \int_{L=\alpha}^{L=\beta} \Gamma_j dL dD.$$

Parameter Estimation

To obtain maximum likelihood estimates for the parameters of our model, we assumed a normal distribution of deviations between the observed (O_{jD}) and predicted (ω_{jD}) mean lengths-

TABLE 3. Maximum-likelihood estimates for parameters of a model proposed to describe the average summer distribution by length and latitude of migrating Pacific hake. Approximate standard errors (SE) were calculated only for the estimated growth parameters and are conditional upon all other estimated parameters being fixed at their maximum-likelihood values.

Parameters for:	Estimate	SE
Migration processes		
Φ	90.37	—
λ_3	0.0523	—
λ_{14}	0.0213	—
K_λ	0.675	—
$D_{s,L=0}$	36.56	—
$D_{s,L=100}$	58.75	—
$D_{s,L=0}$	-36.22	—
$D_{s,L=100}$	-136.60	—
$r_{L=0}$	-0.74	—
$r_{L=100}$	2.92	—
a	0	—
b	0	—
Male growth processes		
l_3	38.96	0.27
l_{14}	53.99	0.16
K_l	0.81	0.01
ψ_3	3.92	0.11
ψ_{14}	2.34	0.24
K_ψ	1.45	0.18
Female growth processes		
l_3	39.64	0.27
l_{14}	57.68	0.15
K_l	0.86	0.01
ψ_3	3.77	0.30
ψ_{14}	4.94	0.07
K_ψ	0.34	0.13
Residual standard error (Eq. 32)		
σ_ω	0.50	—
Residual standard error (Eq. 34)		
σ_ζ	3.52	—

at-age j at latitude D . Thus, minimizing the negative log-likelihood function

$$(32) \quad \Theta_1 = N_\omega \ln[\sqrt{2\pi} \sigma_\omega] + \frac{\sum_{j,D} (O_{jD} - \omega_{jD})^2}{2\sigma_\omega^2}$$

would yield the most probable parameter estimates using only mean lengths-at-age (Edwards 1972). In Eq. 32, σ_ω^2 represents the residual variance and N_ω the number of observed mean lengths-at-age. We used the multinomial negative log-likelihood function

$$(33) \quad \Theta_2 = \sum_{i,j,D} Q_{ijD} \ln \left[\frac{Q_{ijD}}{P_{ijD}} \right], \quad \text{for all } Q_{ijD} > 0$$

to minimize the discrepancy between our observed (Q_{ijD}) and predicted (P_{ijD}) length-at-age frequency data (Edwards 1972; Schnute and Fournier 1980) because our length-at-age frequency data are multinomially distributed. Consequently, minimizing Eq. 33 would yield the most probable parameter estimates using only the length-at-age frequency data.

We also assumed a normal distribution of deviations between the average percentage of hake abundance-at-age in Canadian waters during summer measured by hydroacoustic surveys (C_j)

and that predicted by the model (ζ_j). Thus, minimizing the negative log-likelihood function

$$(34) \quad \Theta_3 = N_\zeta \ln[\sqrt{2\pi} \sigma_\zeta] + \frac{\sum_j (C_j - \zeta_j)^2}{2\sigma_\zeta^2}$$

would yield the most probable parameter estimates based only on the hydroacoustic data. In Eq. 34, σ_ζ^2 represents the residual variance and N_ζ is the number of abundance-at-age measurements.

Our choice was to estimate a 26-parameter vector which included separate growth process parameters for males and females (Table 3), used both the mean length-at-age and length-at-age distribution data sets, and included the hydroacoustic data. Treating the mean length-at-age and length-at-age distribution data independently, and including the hydroacoustic data, means that the most likely estimates are those which minimize the negative log-likelihood function

$$(35) \quad \Theta = \Theta_1 + \Theta_2 + \Theta_3.$$

We used the SIMPLEX algorithm of Nelder and Mead (1965) as implemented by Mittertreiner and Schnute (1985) to minimize the likelihood function Θ , at which point $\Theta_{\min} = \Theta$. During the estimation phase, we found that the parameters a and b both tended toward 0 because a form of Eq. 4 similar to example g in Fig. 3 was most appropriate for our data. We therefore used Eq. A3 instead of Eq. 4, Eq. A4 instead of Eq. 9, and Eq. B16 and Eq. B17 to yield dV_{sL}/dD . Standard errors were calculated only for the estimated male and female growth parameters, and then only with all other estimated parameters set to their maximum-likelihood estimates. Thus the reported standard errors (Table 3) are conditional upon the presumption that all other parameters estimates are equal to the true values for those variables and thus will be optimistic. Standard errors were calculated directly from Eq. 35 using the approximate numerical method supplied with Mittertreiner and Schnute's (1985) SIMPLEX package. This method uses the matrix of second partial derivatives of the likelihood function with respect to all free parameters (calculated numerically) to generate the asymptotic covariance matrix (Kendall and Stuart 1979).

Results

Growth processes were parameterized so that each parameter value would have immediate biological interpretation (Schnute and Fournier 1980; Schnute 1981). A review of the estimates for the male and female growth processes (Table 3; Fig. 6) indicates that both males and females have similar population mean lengths-at-age and population length-at-age variances as 3-yr-olds. After age 3, females grow faster than males and attain larger lengths-at-age than males. A notable result is that the variance in population length-at-age for females increases with age whereas that of males changes little or perhaps decreases slightly with age. This result for females is in contrast with Strait of Georgia hake for which length-at-age variance for both males and females is greatest near age 3 (G. A. McFarlane, unpubl. data). We do not know if this result is related to the migratory nature of this stock, or if it arises from a lack of data in our model on the length-at-age distributions in United States waters.

Unlike the growth processes, the model structure for some migration processes precluded parameterizations which allowed immediate interpretation with respect to the distribution of hake. For example, one might have anticipated estimates for $D_{s,0}$ and

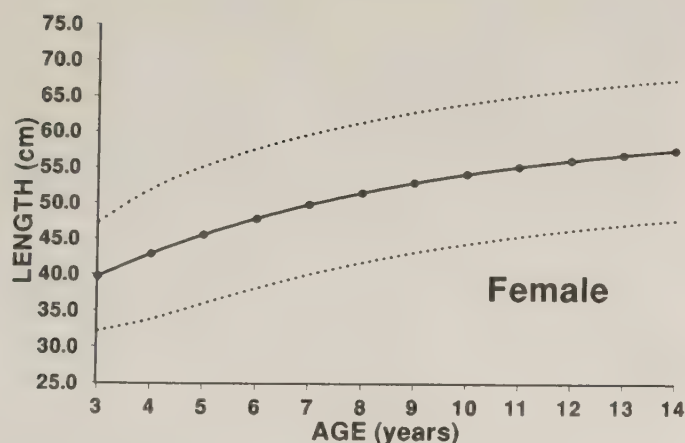
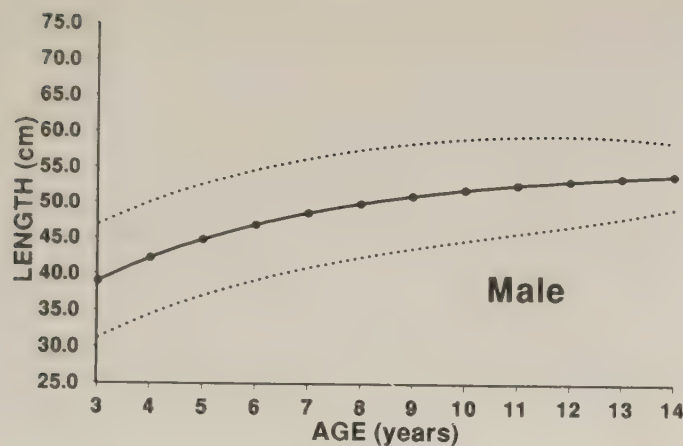


FIG. 6. Predicted von Bertalanffy growth functions describing population mean lengths-at-age for male and female offshore Pacific hake. The dotted lines delimit ± 2 SD of the length-at-age distribution about the mean length-at-age.

$D_{s,100}$ (Table 3) to have values representing latitudes close to those along the migration route of hake. However, this would occur only if at the start of the annual feeding migration, all hake of the same length L were unsegregated by latitude. This condition is never observed in nature because hake are segregated by latitude at all times of year, and consequently also at both extremes of their migration route (Stauffer 1985; Bailey and Francis 1985). Thus, the values for $D_{s,0}$ and $D_{s,100}$ have mathematical interpretation but no useful interpretation with respect to the distribution of hake in nature. This limitation is not serious for our analysis since our interest is the distribution of hake and not the migration parameters per se.

Our model described well the observed changes in sample mean lengths-at-age with latitude with an average deviation (σ) of about 0.5 cm (Table 3; Fig. 7). A sharp increase in sample mean lengths-at-age for younger hake in Canadian waters near 48.7°N is clearly evident for both sexes. This feature results from the accumulation of the larger fish of these age-classes near 48.7°N as they approach the maximum latitude of their migration and begin to slow down (Fig. 8). The right-handed skewness evident in the better sampled length-at-age frequency distributions (Fig. 9) results from the change in migration velocity with latitude as depicted in Fig. 8 which indicates that larger fish tend to travel further north and slow their migration more quickly.

The proportion of hake attaining the more northern latitudes during summer tends to increase with age (Fig. 10). This

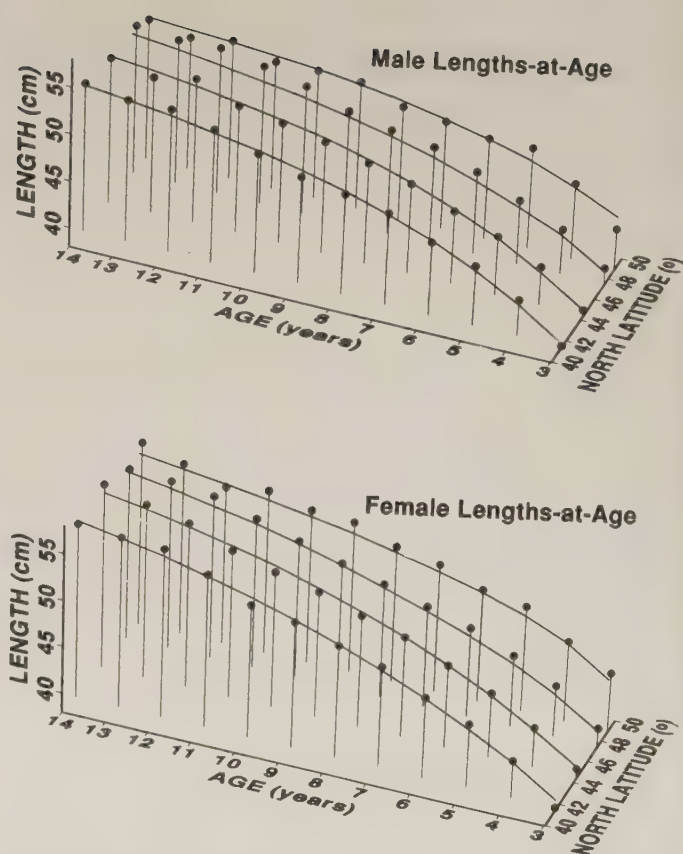


FIG. 7. Observed (O_{ID} , dots) and predicted (ω_{ID} , lines) mean lengths-at-age for male and female offshore Pacific hake samples collected near the latitudes of 41.5, 44.5, 47, and 48.7°N.

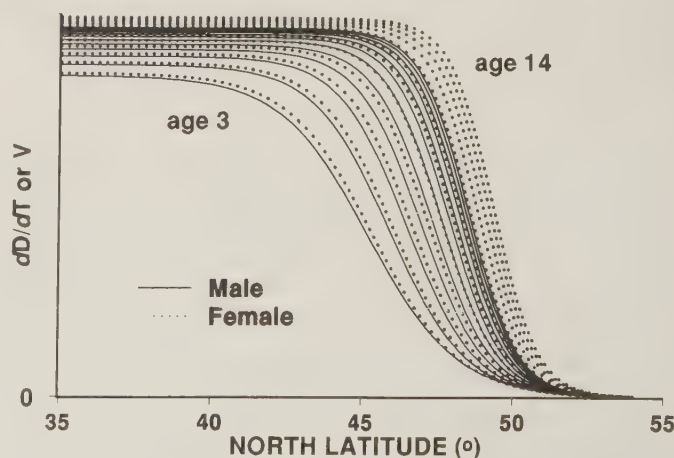


FIG. 8. Estimated relationships between migration velocity (dD/dT or V) and latitude (D , °N) for male and female offshore Pacific hake aged 3–14 yr based on the maximum-likelihood parameter estimates for our migration model (Table 3).

increase is less pronounced for the older age-classes because the length-at-age statistics of the older age-classes become more similar among age-classes. Based on our best parameter estimates, and the assumption of an equal mortality rate for males and females over all age-classes, Fig. 11 presents the average summer percentages of abundance and biomass in Canada, by age, from 1978 until 1988. The estimated summer distributions of abundance of, for example, 3- and 14-yr-old hake (Fig. 12) clearly demonstrate the greater proportion of 14-yr-old hake

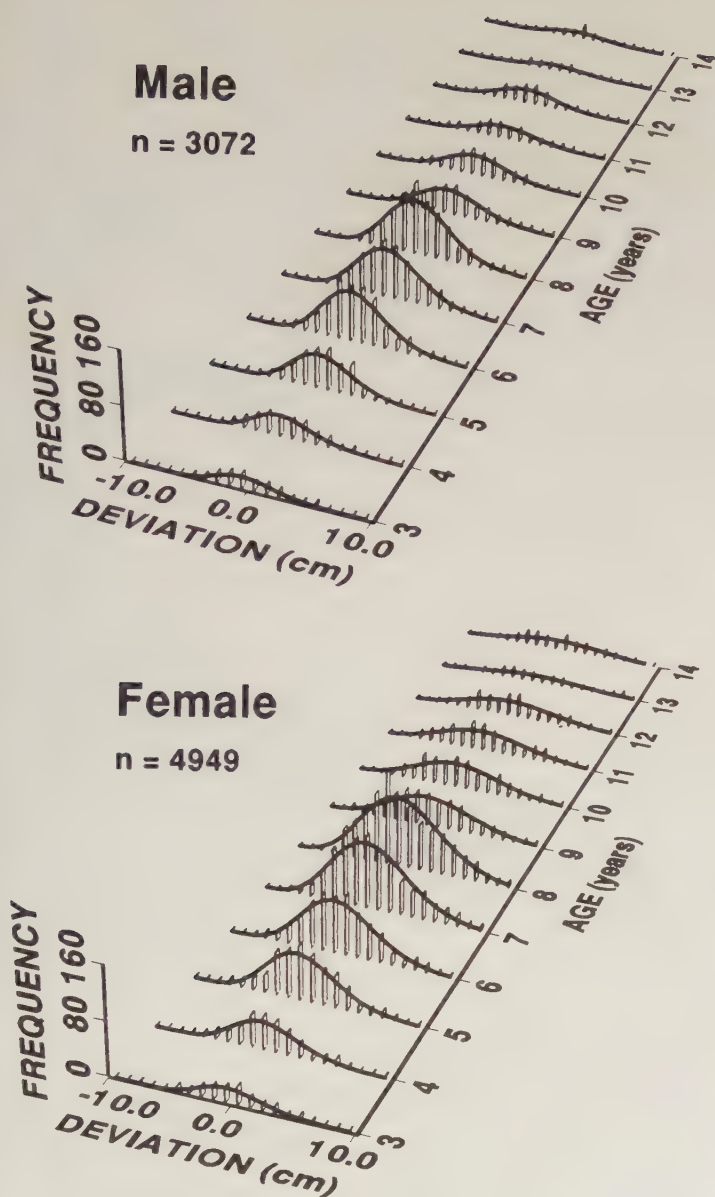


FIG. 9. Observed (Q_{ijD} , histograms) and predicted (P_{ijD} , lines) length-at-age frequency distributions for male (a) and female (b) offshore Pacific hake sampled off southern Vancouver Island near latitude $D = 48.7^\circ\text{N}$. All distributions are reported scaled to a mean of zero. Sample sizes and SD's for the distribution appear in Table 4. Note the right-handed skewness evident in the better sampled age-classes.

in northern waters. The different distributions for males and females reflect the different length-at-age statistics for males and females of the same age (Tables 3 and 4; Fig. 6). Note that the summer abundance of 14^+ -yr-old hake is concentrated near the Canada-U.S. border (Fig. 12); thus, the estimated percentage of older hake in Canadian waters is highly sensitive to the estimate of their latitudinal distribution (Fig. 13).

It is also informative to examine the 1978–88 average summer distribution of the entire Pacific hake population by length and latitude under the simplifying assumptions of a typical natural mortality rate ($M = 0.2$, Dorn and Methot 1990), no fishing, and constant recruitment (Fig. 14). Typically the summer distribution of hake is most heavily concentrated off Washington and southern British Columbia. The characteristic patterns of increased northward migration with increased length, the larger size of females, and a sex ratio favoring females at the more northern latitudes are evident.

TABLE 4. Observed and predicted standard deviations (SD, cm) and sample sizes (n_j) for the male and female frequency distributions of lengths-at-age $j = 3, \dots, 14^+$ shown in Fig. 9.

Age	n_j	Male sd		n_j	Female sd	
		Observed	Predicted		Observed	Predicted
3	99	2.50	2.66	139	2.54	2.61
4	188	2.57	2.50	279	2.66	2.78
5	321	2.36	2.49	503	2.87	2.92
6	494	2.69	2.53	620	3.31	3.09
7	499	2.76	2.59	841	3.41	3.27
8	572	2.59	2.66	942	3.43	3.48
9	268	2.91	2.71	423	3.74	3.68
10	219	2.66	2.74	367	3.68	3.88
11	141	2.96	2.72	310	3.91	4.06
12	154	2.47	2.64	247	4.00	4.22
13	57	2.93	2.47	115	4.81	4.36
14^+	60	2.19	2.15	163	3.90	4.48

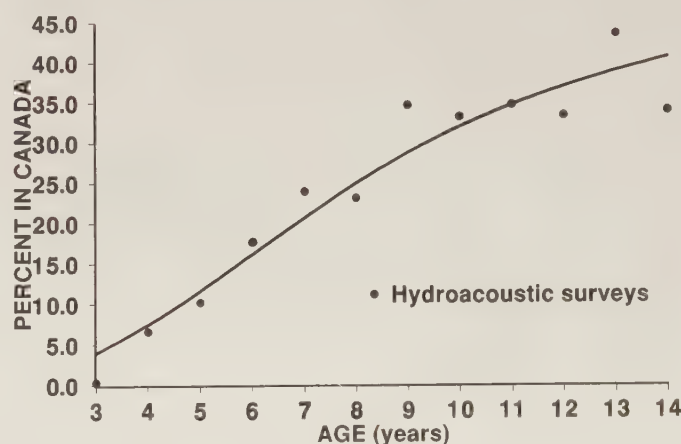


FIG. 10. Average (1978–88) summer percentage of offshore Pacific hake abundance in Canadian waters estimated using our migration model (ζ_j). Points represent the summer percentage abundance-at-age in Canadian waters determined from hydroacoustic surveys (C_j) (Dorn and Methot 1990).

Discussion

Our migration model provides an explanation for the right-handed skewness of length-at-age distributions off southwest Vancouver Island (Fig. 9) and also demonstrates the known tendency (Beamish 1981; Beamish et al. 1982; Barner et al. 1984; Shaw et al. 1987, 1989) for large hake to occur in high abundance off southwest Vancouver Island during summer (Fig. 12 and 14). Furthermore, our approach to the problem of hake abundance suggests an average transboundary distribution of hake abundance (Fig. 14) similar to that suggested by the more established catch-at-age method (fig. 5 of Richards and Saunders 1990) which uses the same assumptions (i.e. $M = 0.2$, constant recruitment, and no fishing). The similarity of our results to those of Richards and Saunders (1990) should be cautiously interpreted, however, because the waters off Vancouver Island have not been adequately surveyed for hake. It is likely that the hydroacoustic surveys of Dorn and Methot (1990) have underestimated the summer abundance of hake in Canadian waters. Recent (1989 and 1990) summer hydroacoustic and trawl surveys in this region (M. W. Saunders, unpubl. data) have located concentrations of large, and predominantly female, hake to the north of the main concentrations

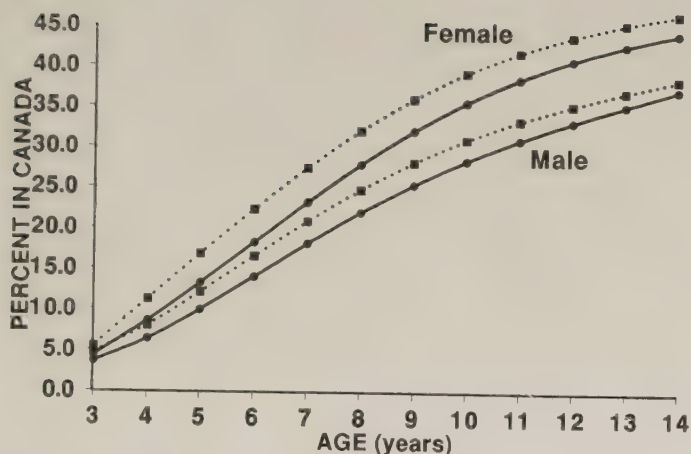


FIG. 11. Estimated average (1978-88) percentage abundance (solid lines) and biomass (assuming $W_L \propto L^{2.73}$ (Frances 1983), dotted lines) of male and female offshore Pacific hake in Canadian waters.

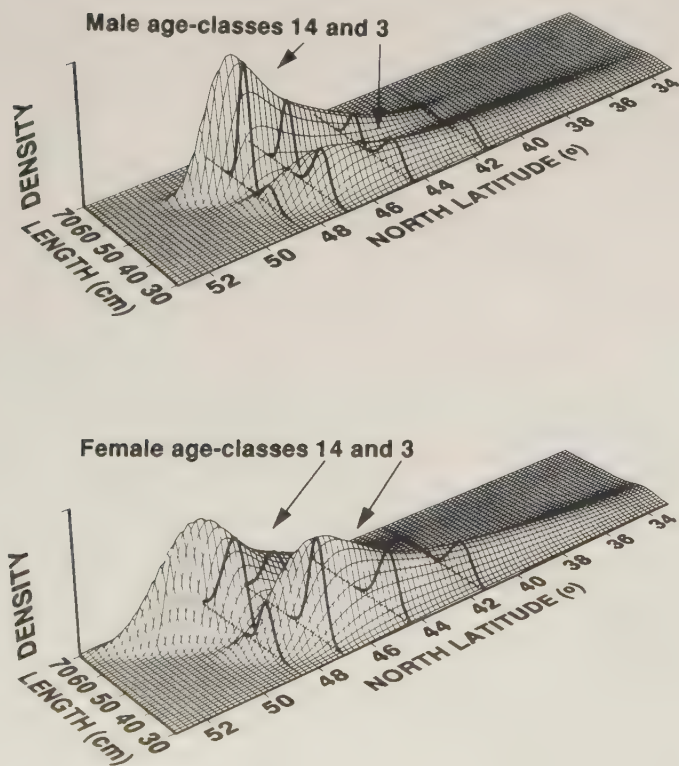


FIG. 12. Average (1978-88) summer distribution of numbers by length and latitude for 3 and 14⁺-yr-old male and female offshore Pacific hake. These depictions were generated using the maximum-likelihood parameter estimates for our migration model (Table 3). The thick solid lines depict the predicted length distributions for these age-classes near the latitudes where hake were sampled.

of hake. Also we routinely hear reports from fishermen of large hake off northern Vancouver Island and off the central coast of British Columbia during winter. Our model results suggest that older and large hake, females in particular, occur to the north of the main fishing area off southern Vancouver Island during summer (Fig. 12-14).

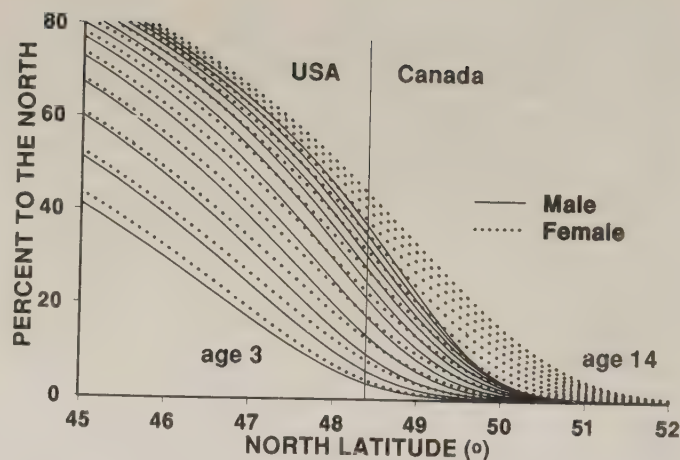


FIG. 13. Average (1978-88) percentage, by age, of male and female offshore Pacific hake estimated to be to the north of the specified latitude during summer from 1978 until 1988.

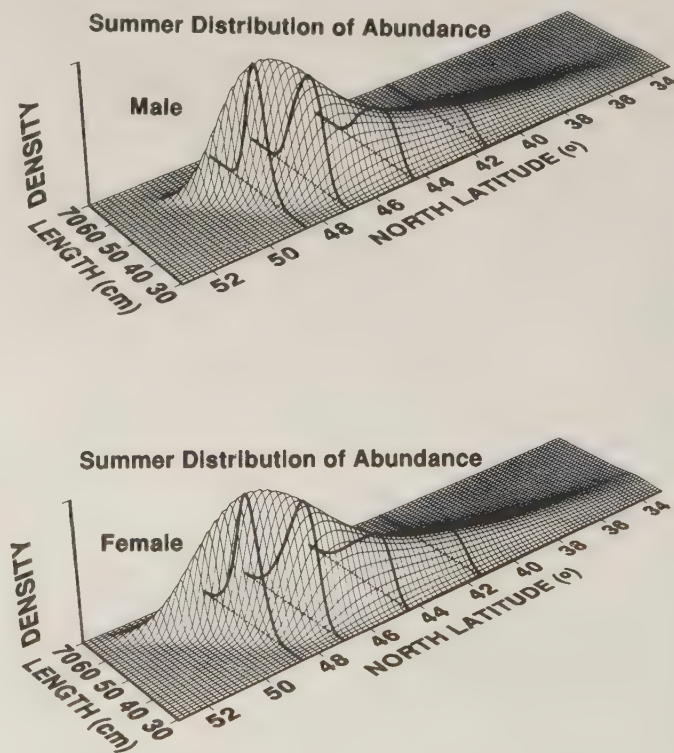


FIG. 14. Average (1978-88) summer distribution of abundance by length and latitude for male and female offshore Pacific hake. This depiction is based on the maximum-likelihood parameter estimates for our migration model (Table 3) and includes ages 3-14⁺ under the assumptions $M = 0.2$, no fishing, and constant recruitment. The thick solid lines depict the length distributions near the latitudes where hake were sampled.

The assessments of Dorn and Methot (1990 and unpubl. data) indicate that when a higher than normal percentage of hake reach Canadian waters during summer, the result is an accumulation of fish off Vancouver Island, and not just a continuous latitudinal shift in distribution with latitude. This is consistent with our model results (Fig. 8) which indicate that hake have substantially slowed their migration velocities by the time they have reached Canadian waters. Also supporting this viewpoint

are the latitudinal series of 1985 length distributions for mature hake in fig. 2 and 3 of Stepanenko (1989). His figures demonstrate a gradual increase in mean lengths of hake in the mature age-classes with increasing latitude until about 46°N. Near this latitude, a more rapid increase in mean lengths begins as the larger individuals slow their migration and accumulate off Washington and Vancouver Island. Our Canadian fishing effort data also support this conclusion. In all years of a Canadian joint-venture fishery the main concentration of trawl sets occurred on the productive shelf area off southern Vancouver Island (as in Fig. 1) where oceanographic conditions promote particularly high productivity (Thomson and Ware 1988; Ware and McFarlane 1989; Thomson et al. 1989).

Our model is therefore a useful tool for conceptualizing the process and patterns associated with the average characteristics of offshore hake migration. However, because dramatic inter-annual variability in year-class strength (Bailey and Francis 1985; Hollowed and Bailey 1989), variable fishing mortality rates (Dorn and Methot 1990), and oceanography influence the latitudinal distribution of hake (Smith et al. 1990), contemporary management of migratory Pacific hake must consider current year-class composition, fishing mortalities, and oceanographic conditions. Thus some important limitations of our model as applied in this analysis are the simplifying assumptions with regard to the condensed data base and a lack of length frequency data from United States waters. Ideally, if our model were used to analyze length-at-age frequency data for several latitudes, at several times of year, and for individual years, then there is the possibility of gaining important insight into hake migration dynamics, the distribution and abundance of hake, and the degree and nature of the marked interannual variability in these processes and patterns (Methot 1989; Dorn and Methot 1990; Smith et al. 1990).

For example, the most recent stock assessment (M. W. Dorn and R. D. Methot, unpubl. data) suggests that the percentage summer transboundary split of the older age-classes (Canada:U.S.) might vary from 32.68 to 56.44 depending on the year. The highest recorded summer abundance of hake in Canadian waters occurred during the 1983 El Niño year when stronger than normal coastal surface currents assisted the northward migration of hake (Mysak 1986; Smith et al. 1990). The California Undercurrent within which Pacific hake reside is typically characterized by northward current velocities during the spring of about 4–5 km per day (Lynn and Simpson 1987). During the spring of 1983, average current velocities reached ≈30 km per day for the northward flowing Davidson Current (Mysak 1986) at which time it is essentially a component the California Undercurrent (Lynn and Simpson 1987). Consequently, our model could be used to increase our understanding of the effects of interannual variability in oceanographic conditions on length-at-age statistics (Smith et al. 1990), as well as the latitudinal distribution and transboundary split of hake. These questions about Pacific hake population dynamics could also be relevant to other species of hake, such as Argentine hake (*Merluccius hubbsi*) in the southwestern Atlantic Ocean (Podestá 1990).

Finally, we note the value of the length-at-age frequency data for our analysis. Although the use of length frequency distributions for analyzing growth patterns is common, our work suggests that some length frequency distributions might contain much more information than suspected. Frequency distributions are generally based on hundreds, if not thousands, of data points; thus, the information they contain tends to be statistically reliable. For example, our conclusions are based on some-

what undramatic skewness in frequency distributions and small trends in mean lengths-at-age. In comparison with other types of sampling data (e.g. direct measurements and summary statistics), analyses of frequency distributions appear less often in fisheries literature. We suspect that the archives of many fisheries institutions store large quantities of frequency data, and we suggest that researchers be alert to the potential for increasing our understanding of population processes by appropriate analysis of informative frequency distributions.

Acknowledgments

We are particularly grateful to our colleagues R. Methot and M. Dorn for providing us with the mean length-at-age data collected from United States waters which were used in this analysis. The content and presentation of this manuscript were also improved through criticisms by Drs. J. C. Rice and D. W. Welch of the Pacific Biological Station.

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Appendix A

Solutions for the integration of Eq. 4 over D and T for the initial condition of $D = D_{sl}$ at $T = 0$, and for the three limiting conditions $a > 0, b = 0$; $a = 0, b > 0$, and $a = 0, b = 0$.

When $a > 0, b > 0$, the solution is Eq. 9.

When $a > 0, b = 0$, the solution is

$$(A1) \quad \left\{ \frac{(V_{*L} + 2a)\tau_2}{r_L(V_{*L} + a)^2} \right\} + T = 0.$$

When $a = 0, b > 0$, the solution is

$$(A2) \quad \left\{ \frac{r_L(V_{*L} - b)(D - D_{sl}) + b(e^{r_L(D - D_{*L})} - e^{r_L(D_{sl} - D_{*L})})}{r_L V_{*L}^2} \right\} - T = 0.$$

When $a = 0, b = 0$, Eq. 4 is reparameterized as

$$(A3) \quad V_{DL} = \frac{2V_{*L}e^{-r_L(D - D_{*L})}}{1 + e^{-r_L(D - D_{*L})}}$$

to assure the statistical stability of V_{*L} and maintain $V_{DL} = V_{*L}$ at $D = D_{*L}$. When integrated over D and T and set to the initial condition of $D = D_{sl}$ at $T = 0$, the solution is

$$(A4) \quad \left\{ \frac{r_L(D - D_{sl}) + e^{r_L(D - D_{*L})} - e^{r_L(D_{sl} - D_{*L})}}{2r_L V_{*L}} \right\} - T = 0.$$

Appendix B

Solutions to the derivative dV_{*L}/dD of Eq. 12 for the four conditions $a > 0, b > 0$; $a > 0, b = 0$; $a = 0, b > 0$; and $a = 0, b = 0$. By the chain rule:

$$(B1) \quad \frac{dV_{*L}}{dD} = -\frac{\partial F}{\partial D} \frac{\partial V_{*L}}{\partial F}$$

where F represents Eq. 9 when $a > 0, b > 0$ and F represents

Eq. A1, A2, or A4 for the limiting conditions $a > 0, b = 0$; $a = 0, b > 0$; or $a = 0, b = 0$, respectively.

When $a > 0, b > 0$:

$$(B2) \quad \frac{\partial F}{\partial D} = - \left\{ \frac{e^{-r_L(D-D_{*L})} (V_{*L} + 2a - b) + b}{e^{-r_L(D-D_{*L})} (V_{*L} + a)^2 - a^2} \right\}.$$

For clarity and ease of programming, the derivative $\partial F/\partial V_{*L}$ is represented as the sum of intermediate terms τ_3 – τ_6 :

$$(B3) \quad \frac{\partial F}{\partial V_{*L}} = \tau_3 - \tau_4 + \tau_5 - \tau_6$$

where

$$(B4) \quad \tau_3 = \frac{2(2a^3 + a^2V_{*L} + 2abV_{*L} + bV_{*L}^2) e^{-r_L(D-D_{*L})}}{a^2r_L(V_{*L} + a) (e^{-r_L(D-D_{*L})} (V_{*L} + a)^2 - a^2)}$$

$$(B5) \quad \tau_4 = \frac{2(2a^3 + a^2V_{*L} + 2abV_{*L} + bV_{*L}^2) e^{-r_L(D_{sL}-D_{*L})}}{a^2r_L(V_{*L} + a) (e^{-r_L(D_{sL}-D_{*L})} (V_{*L} + a)^2 - a^2)}$$

$$(B6) \quad \tau_5 = \frac{(a^2 + 2ab + 2bV_{*L}) \tau_2}{a^2r_L(V_{*L} + a)^2}$$

$$(B7) \quad \tau_6 = \frac{2(2a^3 + a^2V_{*L} + 2abV_{*L} + 2bV_{*L})\tau_2}{a^2r_L(V_{*L} + a)^3}$$

When $a > 0, b = 0$:

$$(B8) \quad \frac{\partial F}{\partial D} = - \left\{ \frac{e^{-r_L(D-D_{*L})} (V_{*L} + 2a)}{e^{-r_L(D-D_{*L})} (V_{*L} + a)^2 - a^2} \right\}.$$

For clarity and ease of programming, the derivative $\partial F/\partial V_{*L}$ is represented as the sum of intermediate terms τ_7 – τ_{10} :

$$(B9) \quad \frac{\partial F}{\partial V_{*L}} = \tau_7 - \tau_8 + \tau_9 - \tau_{10}$$

where

$$(B10) \quad \tau_7 = \frac{2(V_{*L} + 2a)e^{-r_L(D-D_{*L})}}{r_L(V_{*L} + a)(e^{-r_L(D-D_{*L})} (V_{*L} + a)^2 - a^2)}$$

$$(B11) \quad \tau_8 = \frac{2(V_{*L} + 2a)e^{-r_L(D_{sL}-D_{*L})}}{r_L(V_{*L} + a)(e^{-r_L(D_{sL}-D_{*L})} (V_{*L} + a)^2 - a^2)}$$

$$(B12) \quad \tau_9 = \frac{\tau_2}{r_L(V_{*L} + a)^2}$$

$$(B13) \quad \tau_{10} = \frac{2(V_{*L} + 2a) \tau_2}{r_L(V_{*L} + a)^3}.$$

When $a = 0, b > 0$:

$$(B14) \quad \frac{\partial F}{\partial D} = \frac{(V_{*L} - b) + b e^{r_L(D-D_{*L})}}{V_{*L}^2}$$

$$(B15) \quad \frac{\partial F}{\partial V_{*L}} = \frac{r_L(D-D_{sL})(b-2V_{*L}) - 2b(e^{r_L(D-D_{*L})} - e^{r_L(D_{sL}-D_{*L})})}{r_L V_{*L}^3}.$$

When $a = 0, b = 0$:

$$(B16) \quad \frac{\partial F}{\partial D} = \frac{1 + e^{r_L(D-D_{*L})}}{2V_{*L}}$$

$$(B17) \quad \frac{\partial F}{\partial V_{*L}} = - \left\{ \frac{r_L(D-D_{sL}) + e^{r_L(D-D_{*L})} - e^{r_L(D_{sL}-D_{*L})}}{2r_L V_{*L}^2} \right\}.$$

Environmental Fate of Polychlorinated Dibenzo-*p*-dioxins in Lake Enclosures

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The environmental fate of 1,3,6,8-tetra- (T_4 CDD) and octachlorodibenzo-*p*-dioxin (O_8 CDD), two major dioxin congeners emitted into the environment, was studied in large (40 m³) lake enclosures at the Experimental Lakes Area in northwestern Ontario. The polychlorinated dioxins (PCDDs) were added to replicate enclosures as a sediment slurry at a nominal concentration of 58–59 ng·L⁻¹. Both congeners partitioned/settled rapidly to the surficial sediments where they persisted over the 2 yr of the study. Initially the concentrations of the T_4 CDD in water were higher than those of O_8 CDD, but the concentrations of the T_4 CDD in the water column declined more rapidly than those of O_8 CDD, with $t_{1/2}$ of 2.6 ± 0.2 and 4.0 ± 0.3 d, respectively. Approximately 10–15% of the T_4 CDD and <1% of the O_8 CDD detected in the water column during the first 48 h were determined to be truly dissolved. The rapid partitioning of O_8 CDD and to a lesser extent T_4 CDD to dissolved and particulate organic matter in the water column and sediments limited their bioavailability. Increased retentive capacity of the higher chlorinated PCDDs may explain the pattern of increasing concentration of PCDDs in sediments with increasing chlorine substitution observed in the Great Lakes and other aquatic environments.

Le devenir environnemental de la 1,3,6,8-tétra- (T_4 CDD) et de l'octachlorodibenzo-*p*-dioxine (O_8 CDD), deux importantes congénères de la dioxine libérés dans l'environnement, ont été étudiés dans de grandes enceintes (40 m³) de la région des lacs expérimentaux, dans le nord-ouest de l'Ontario. On a ajouté les dioxines polychlorées (PCDD) sous forme de boues de sédiments dans plusieurs enceintes identiques, à une concentration nominale de 58–59 ng·L⁻¹. Les deux congénères se sont séparés et se sont déposés rapidement sur les sédiments superficiels, où ils ont persisté pendant les 2 ans de l'étude. Au début, les concentrations de T_4 CDD dans l'eau étaient supérieures à celles d' O_8 CDD, mais les concentrations de T_4 CDD dans la colonne d'eau ont diminué plus rapidement que celles d' O_8 CDD, avec des $t_{1/2}$ de $2,6 \pm 0,2$ et de $4,0 \pm 0,3$ jours, respectivement. Environ 10–15 % de la T_4 CDD et moins de 1 % de l' O_8 CDD décelé dans la colonne d'eau au cours des premières 48 h étaient vraiment en solution, d'après les mesures. La séparation rapide de l' O_8 CDD et, dans une moindre mesure, celle de la T_4 CDD entre la solution et les matières particulaires organiques et sédimentaires dans la colonne d'eau limitaient la biodisponibilité. Une capacité de rétention accrue des PCDD plus fortement chlorés peut expliquer le profil des concentrations des PCDD dans les sédiments, qui augmentent avec la substitution par le chlore, qu'on a observé dans les Grands Lacs et d'autres milieux aquatiques.

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Polychlorinated dibenzo-*p*-dioxins (PCDDs) are a large group (75 congeners) of potentially hazardous environmental contaminants. Concern about the presence of these compounds in aquatic environments arises from the extremely high toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8- T_4 CDD) to fish (Mehrlle et al. 1988) and mammals (Esposito et al. 1980) and the potential for biomagnification of 2,3,7,8-substituted congeners in food chains leading to human consumers (Kuehl et al. 1987; De Vault et al. 1989). Non-2,3,7,8-substituted dioxins are of less concern from the point of mammalian toxicity (Barnes et al. 1986; Esposito et al. 1980) but they are emitted to the environment in relatively large quantities, especially via combustion processes such as waste incineration and burning of leaded gasoline (Rappe et al. 1987;

Ballschmitter et al. 1986). Concentrations of PCDDs found in sediments from the Great Lakes (Czuczwa and Hites 1986) and from German lakes (Hagenmaier et al. 1986) increase with increasing chlorine substitution. Surficial sediments (0–1 cm) in Lake Huron contained <1 pg total tetrachloro homologs·g⁻¹ and concentrations of octachlorodioxin (O_8 CDD) ranging as high as 1300 pg·g⁻¹ (Czuczwa and Hites 1986). Little is known, however, about the environmental fate of PCDDs, other than 2,3,7,8- T_4 CDD, in aquatic environments.

This study examines the environmental fate of O_8 CDD and 1,3,6,8-tetrachlorodibenzo-*p*-dioxins (T_4 CDD) in the aquatic environment. T_4 CDD was selected for the study because it is the dominant tetrachloro- congener in incinerator fly ash (Yasuhara et al. 1987; Kuehl et al. 1985) and auto exhaust (Ballschmitter et al. 1986) and a major dioxin contaminant of various 2,4-D (ester) and diphenyl ether herbicide formulations (Cochrane et al. 1980; Tamagishi et al. 1981). The 1,3,6,8-congener has similar physical properties to 2,3,7,8- T_4 CDD

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(Shiu et al. 1988) and has been shown to behave in a manner similar to the latter congener following addition to small outdoor ponds (Corbet et al. 1988; Tsushimoto et al. 1982). The 1,3,6,8- T_4 CDD congener is therefore of interest as a major tetrachlorodioxin emitted to the environment and as a surrogate for the study of the fate of the more toxic 2,3,7,8- T_4 CDD. Octachlorodibenzo-*p*-dioxin (O_8 CDD) was selected for study because it is the most hydrophobic PCDD congener and one of the most abundant dioxin contaminants in fly ash, pentachlorophenol, air particulates, and Great Lakes sediments (Czuczwa and Hites 1986).

The objective of the study was to examine the environmental behaviour of these two PCDDs, which represent the extremes in physical/chemical properties of this class of compounds, under natural field conditions simulating a lake environment. The present paper describes the environmental fate of the two compounds following their addition to 5-m diameter mesocosms in Lake 304 of the Experimental Lakes Area. A companion paper describes the bioavailability of the compounds (Servos et al. 1992).

Methods

Chemical Analyses

Uniformly ring-labelled [^{14}C] T_4 CDD and [^{14}C] O_8 CDD were purchased from Pathfinder Laboratories (St. Louis, MO) and had specific activities of 894 and 761 MBq·mmol $^{-1}$, respectively. Both congeners were determined to be >99.8% radiochemically pure by high-performance liquid chromatography (HPLC) using conditions described in detail below. Stock solutions were made up of 3.92 mg in 50 mL of tetrahydrofuran. Tritiated water was supplied by New England Nuclear (Boston, MA). ^{14}C and 3H activities were determined by diluting environmental samples or extracts with scintillation fluor (Atomlight, New England Nuclear) and assaying by liquid scintillation counting (LSC). ^{14}C and 3H activities were determined simultaneously using a dual-label calculation routine on a Beckman LC 7500 with automatic quench compensation (Beckman Instruments, Irvine, CA). Selected samples were combusted using a Packard 306 sample oxidizer (Packard Instruments Co., Downers Grove, IL). The $^{14}CO_2$ was trapped in 2-methoxyethylamine, diluted with scintillation fluor, and assayed by LSC.

HPLC was performed using a Waters model 6000A solvent delivery system and a μ Bondapak C_{18} column (Waters Scientific, Milford, MA). The mobile phase was methanol, and 0.5-min fractions were collected and assayed by LSC. The T_4 CDD and O_8 CDD had retention times of 4.3 and 6.2 min, respectively.

Mesocosms

Five 5 m diameter $\times$ 2 m deep enclosures were constructed in the littoral zone of Lake 304 at the Experimental Lakes Area in northwestern Ontario (Johnson and Vallentyne 1971). Sediments collected from Lake 304 at 2 m had a clayey texture with 12% sand, 37% silt, and 51% clay. Sediments contained 97.5% water and were 25.2% dry weight total organic carbon and 2.4% dry weight total nitrogen. Dissolved organic carbon (DOC) in the sediment interstitial water was 28.8 ± 3.6 mg·L $^{-1}$. The water and surface-sediment chemistry of Lake 304 has been described in more detail previously (Armstrong and Schindler 1971; Brunskill and Schindler 1971; Brunskill et al. 1971). The chemical and physical characteristics of the lake and enclosures are summarized in Table 1.

TABLE 1. Chemical characteristics of enclosure and lake water prior to the addition of polychlorinated dioxins (May 15 – June 11, 1985).

	Enclosure		Lake	
	Mean $\pm$ SD	N	Mean $\pm$ SD	N
Suspended P (μ g·L $^{-1}$)	11 $\pm$ 2	20	10 $\pm$ 2	6
Suspended N (μ g·L $^{-1}$)	125 $\pm$ 15	20	106 $\pm$ 8	6
Suspended C (μ g·L $^{-1}$)	1110 $\pm$ 128	20	997 $\pm$ 67	6
DOC (mg·L $^{-1}$)	7.8 $\pm$ 0.6	20	8.9 $\pm$ 0.3	6
Conductivity (μ mho)	19 $\pm$ 1	20	20 $\pm$ 1	6
Temperature ($^{\circ}C$)	16 $\pm$ 1	20	16 $\pm$ 1	6
Alkalinity (μ eq·L $^{-1}$)	95 $\pm$ 4	4	85	1

Enclosures were constructed of polyethylene reinforced with nylon and supported by a tubular frame floated on the surface using styrofoam blocks inside a pocket sewn into the top edge. The enclosure tubes were sunk into the soft bottom sediments using heavy chain and angle iron held inside folds sewn into the bottom edges. A SCUBA diver then pushed the bottoms of the corrals into the sediments as far as possible, at least 30 cm. The enclosures were constructed and put into place May 8–11, 1985, and allowed to equilibrate for approximately 4 wk.

Chemical Additions

On June 12, PCDDs were added to the mesocosms as a sediment slurry. Because of the extremely hydrophobic nature of PCDDs, they primarily enter or exist in the environment associated with organic particles. PCDDs entering aquatic environments would also quickly become associated with organic particles in the water column. Sorbing the PCDDs to sediments was therefore a realistic and practical way to introduce these chemicals to the enclosures. Sorbing the PCDDs to sediments also prevented a large fraction of the PCDDs from sorbing to the enclosure walls.

The stock solutions (3.92 mg of each compound in tetrahydrofuran) were transferred into 4-L glass bottles which contained approximately 0.5 kg wet weight (12.5 g dry weight) sediment collected from the littoral zone of Lake 304 (2 m). The bottles were shaken repeatedly for 3–4 h prior to addition to the lake enclosures. The PCDD congeners were mixed into the enclosures by slowly (15–20 min) pouring the sediment from the bottle into the propeller wash of an electric outboard motor. Two enclosures were treated with the T_4 CDD, two with the O_8 CDD, and one served as a control. A site approximately 10 m away from the enclosures served as a lake reference. Seventy-four becquerels of tritiated water was added to each enclosure (with the sediment slurry) to determine leakage and to estimate the total volume at the beginning of the experiments. Eight 4-mL water samples were taken from each enclosure on day 1, assayed by LSC, and the volume of each enclosure estimated.

Water

A 4-L water sample was collected from the centre of each corral at 1 m depth at each sampling date. One litre (days 0–9) or 4 L of unfiltered water was extracted twice with 200 mL of dichloromethane (DCM) in either a 1-L shake flask or a 4-L bottle with constant stirring for 30 min. All extracts were dried on approximately 50 g of anhydrous Na_2SO_4 , rotoevaporated to 1 mL, and subsamples counted by LSC. Selected extracts were also assayed by HPLC as described above. The extraction efficiency was determined (on day 1) to be 78 ± 8 and $77 \pm 5\%$ (means $\pm$ SD) for the T_4 CDD and O_8 CDD, respectively.

During the first 72 h, additional water samples were also taken to determine the partitioning of PCDDs among particulate organic matter (POM), dissolved organic matter (DOM), and that truly dissolved in solution ("free"). Replicate 4-mL samples of whole water were counted directly by LSC. Replicate 25-mL whole-water samples were centrifuged for 30 min at 20 000g in Corex glass centrifuge tubes. A 4-mL aliquot of the supernatant was assayed directly by LSC to determine the proportion of radioactivity "in solution." The proportion of radioactivity associated with the DOM was determined using a method described by Landrum et al. (1984). A 4-mL aliquot of the supernatant was passed through a reverse-phase cartridge (C₁₈ Sep-Pak, Waters Scientific) and the eluant assayed by LSC. The PCDD associated with the DOM partitions to the C₁₈ and remains on the column (Landrum et al. 1984). Half-lives ($\pm$ SD) of PCDDs in water and other compartments were estimated by linear regression (SAS-GLM) assuming a pseudo-first-order kinetic rate model (SAS Institute Inc. 1982).

Surface Films

The surface film was collected using a technique similar to that described by Harvey and Burzell (1972). A 20 $\times$ 20 cm glass plate was lowered horizontally until it touched the surface of the water, and then the plate was lifted and washed with DCM. DCM was reduced to 0.5 mL and aliquots of both the water and DCM were assayed by LSC. Glass plate samplers collected a 0.56 ± 0.04 mm layer of water.

Particulate Organic Matter >100 μ m (Zooplankton)

A 30 cm diameter Wisconsin net (100 μ m) was pulled vertically through the top 1.5 m of each enclosure and washed in the adjacent lake. The contents were transferred to glass jars and frozen at -50°C for later analysis. Samples were filtered through No. 40 ashless Whatman filters (Whatman, Maidstone, England) and combusted on a sample oxidizer.

Periphyton/Polyethylene

The polyethylene presented a very large potential sink for the PCDDs. In order to quantify the mass of PCDDs in this compartment, strips of the polyethylene wall material 2.5 mm $\times$ 2 m were covered with packing tape on one side and hung from the north side of each enclosure 3 wk prior to the "spike" date. On each sampling date, two strips were collected by rolling the strip under the surface of the water while removing the tape backing. Each strip was separated into 0.5-m sections. Samples were transferred into separate glass jars and frozen until analyzed. The strips were scraped with a glass slide and the periphyton collected on No. 40 ashless Whatman filters and combusted on a sample oxidizer. After day 14, periphyton was collected directly from the walls by scraping with glass slides in situ. On the "spike" date, additional polyethylene strips were added to each enclosure. On each sampling date, these strips were collected as described above. The strips served as a comparison for the effect of colonization on the sorption of PCDDs. Duplicate 2-cm pieces of polyethylene were cut from the centre of each section of the strips collected (i.e. 0.25, 0.75, 1.25 m depth). The polyethylene pieces were placed into scintillation vials, diluted with scintillation fluor, and assayed directly by LSC. There was no significant relationship of ^{14}C concentration with depth; therefore, all samples on each date were pooled for comparisons.

Sediment

Jars

Sediment was collected from Lake 304 at a depth of 2 m, using an Ekman dredge, 3 d prior to the spike date. It was placed into 7 cm diameter $\times$ 8 cm deep glass jars such that each jar was approximately two thirds full. Jars were lowered into each enclosure, attached to strings so that they could be easily retrieved without disturbing the sediments. On each sampling date (up to 24 d, jars were collected and immediately capped and frozen until analyzed. Sediments were freeze-dried and then thoroughly mixed and a 3-g aliquot refluxed in 1:1 hexane-acetone for 24 h. The slurry was filtered through a Whatman GFC filter, the eluent rotoevaporated to approximately 10 mL, further reduced to 2 mL under a stream of N₂, and then an aliquot assayed by LSC. Aliquots of the extracts were also analyzed by HPLC to determine the percentage of each congener remaining as the parent compound. Triplicate samples (approximately 0.10 g each) of both the extracted and unextracted sediments were combusted on a sample oxidizer to determine the total ^{14}C .

Recovery of T₄CDD and O₈CDD spiked onto dried sediments was 77.6 ± 5.8 and $78.8 \pm 5.2\%$, respectively. To determine if there were significant losses of PCDDs during freeze-drying, 10 wet sediment samples (0.5 g each of sediment filtered through a Whatman GFC filter) were spiked with each congener. Half of the samples were freeze-dried and then combusted and the other half were combusted directly. Mean recovery for T₄CDD and O₈CDD after freeze-drying was 103 ± 3 and $109 \pm 11\%$, respectively.

Cores

Duplicate core samples of the sediment were taken with a 5 cm diameter KB corer. Water was siphoned off and then the sediment pushed up the core tube and the 0–6 cm fraction collected. Sediments were treated and analyzed as described for sediment jars. Fewer sediment core samples were collected during the first 24 d than sediment jar samples, and only a single core was taken from each enclosure on day 3 to reduce the probability of disturbing the water column.

Profiles

Sediment core samples were collected as described above but the sediments were cut into 2-cm slices and transferred into glass jars. To avoid contamination from the layer above, a 2.5-cm core was taken of the inside of each core slice. Samples were transferred into 25-mL Corex glass centrifuge tubes and centrifuged for 15 min at 20 000 g. The supernatant was taken off and recentrifuged for 30 min at 20 000 g. A 4-mL sample of the supernatant was assayed for both ^{14}C and ^3H . The pellet was transferred back to the sample jars and frozen until analyzed. The sediment was freeze-dried and two subsamples (0.10 g) combusted on a sample oxidizer.

Modelling

A microcomputer spreadsheet version of the Quantitative Water Air Sediment Interaction Model (QWASI) described by Southwood et al. (1989) was used to predict the relative importance of different removal processes occurring in the mesocosms. QWASI is an unsteady-state fugacity-based model developed to describe the fate of chemicals in aquatic systems (Mackay et al. 1983). The chemical parameters required for the model were collected from the literature (Shiu et al. 1988; Choudhry and Webster 1988). An aquatic system simulating as closely as possible the enclosures was used in the model

TABLE 2. Primary inputs and process calculation output for the QWASI model simulating a single spike of each PCDD to a lake enclosure.

	T ₄ CDD	O ₈ CDD
Physical properties		
Molecular mass (g·mol ⁻¹)	321.9	459.8
Vapor pressure (Pa)	5.8×10^{-5}	1.2×10^{-7}
Solubility (mol·m ⁻³)	8.2×10^{-5}	1.4×10^{-7}
Log K _{ow}	7.1	8.2
Inputs for calculation of Z		
Temperature (K)	298	298
% organic carbon in sediment	25.2	25.2
Density of biota (kg·L ⁻¹)	1	1
Density of suspended sediment (kg·L ⁻¹)	1.3	1.3
Density of sediment (kg·L ⁻¹)	1.3	1.3
Volumes (m³)		
Water	67	67
Sediment	1.2	1.2
Suspended sediment	1×10^{-4}	1×10^{-4}
Biota	4×10^{-4}	4×10^{-4}
Areas (m²)		
Air–water interface	20	20
Sediment–water interface	20	20
Mass transfer coefficients (m·h⁻¹)		
Air-side MTC	5.0	4.2
Water-side MTC	6.6×10^{-3}	5.6×10^{-3}
Rate constants (h⁻¹)		
Transformation in sediment	0	0
Transformation in sediment (<i>t</i> _{1/2} = 10 yr)	7.9×10^{-6}	7.9×10^{-6}
Transformation in water	9.4×10^{-2}	1.6×10^{-3}
Other inputs		
Path length for diffusion (m)	1.5×10^{-2}	1.5×10^{-2}
Diffusivity (m ² ·h ⁻¹)	1×10^{-6}	1×10^{-6}
G values (m³·h⁻¹)		
Burial	6.4×10^{-7}	6.4×10^{-7}
Resuspension	1.3×10^{-7}	1.3×10^{-7}
Emissions (mol·h⁻¹)		
	0	0
Initial concentrations		
Water (μg·L ⁻¹)	0.01	0.01
Sediment (ng·g dry wt ⁻¹)	100	100
Process calculations (mol·h⁻¹·Pa⁻¹)		
Sediment burial	1.5	16.1
Sediment transformation	0	0
Sediment transformation (<i>t</i> _{1/2} = 10 yr)	22.7	237
Water transformation (photolysis)	9.0	0.1
Water to air volatilization	0.03	0.03

(Table 2). The model integrates the many processes occurring in the environment and provides a framework from which to examine the laboratory and field results.

Results

Concentrations (total ¹⁴C) of T₄CDD in whole-water samples were higher than those of O₈CDD until day 2 but declined rapidly with a first-order half-life of 2.6 ± 0.2 d ($R^2 = 0.097$) compared with a half-life for O₈CDD of 4.0 ± 0.3 d ($R^2 = 0.96$) (Fig. 1). The concentration of O₈CDD remained higher than that of T₄CDD after day 3 until the termination of the experiment. During the first 48 h, 10–15% of the T₄CDD and <1% of the O₈CDD were extractable by C₁₈ Sep-Paks (Fig. 1). Approximately 10–30% of both PCDDs were associated with DOC (unextractable with C₁₈ Sep-Pak), while the remaining 75–90% was associated with particulate organic carbon (POC) (centrifugable).

The surface films contained both T₄CDD and O₈CDD concentrations (total ¹⁴C) 4–18 times higher than in the water columns. The concentrations of the O₈CDD were 2–20 times higher than the concentrations of T₄CDD (Fig. 2). The concentrations of O₈CDD remained high ($125\text{--}220$ ng·L⁻¹) for the first 4 d and then declined rapidly with a half-life of 1.3 ± 0.2 d ($R^2 = 0.74$).

The initial concentration (day 1) of T₄CDD (total ¹⁴C) in POM > 100 μm (zooplankton) was 3.8 times higher than the concentration of O₈CDD (Fig. 3). The concentration of T₄CDD in POM declined rapidly but concentrations remained between 20 and 67 ng·g⁻¹ (dry weight) after day 9, with the exception of day 54 when concentrations rose to 108–248 ng·g⁻¹.

Unlike POM, the concentrations of T₄CDD and O₈CDD (total ¹⁴C) in periphyton increased gradually with time until 14–103 d (Fig. 4). The concentrations of O₈CDD were as much as 25 times higher than those of T₄CDD. After day 24 the O₈CDD

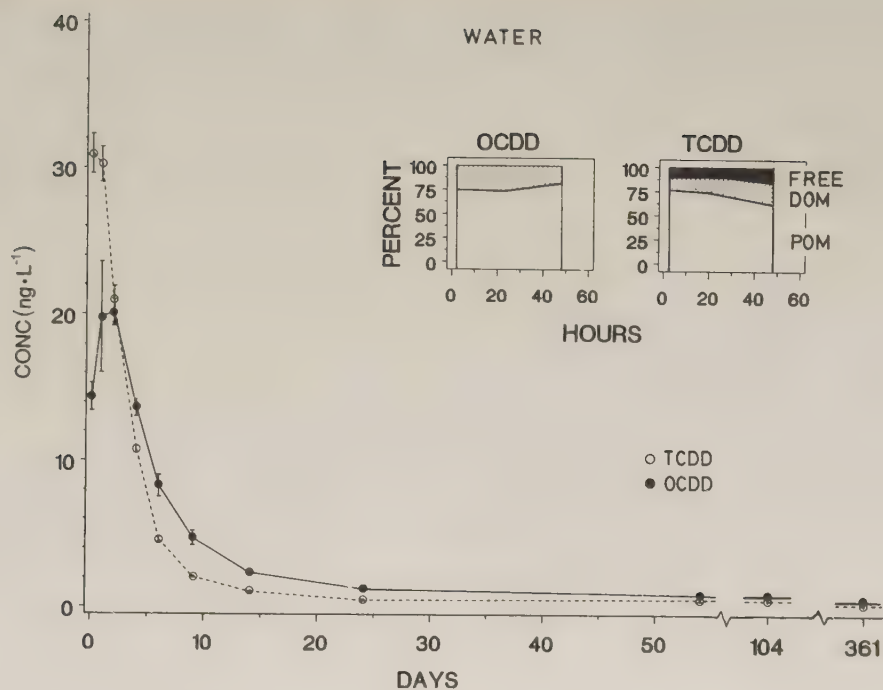


FIG. 1. Total water concentration of PCDDs (extractable ^{14}C) in lake enclosures. Bars represent the range in the enclosure means. Nominal concentrations in the water were 59.0 and $58.4 \text{ ng}\cdot\text{L}^{-1}$ for T_4CDD and O_8CDD , respectively. Insert represents the percentage of the ^{14}C associated with POM or DOM or in true solution (free).

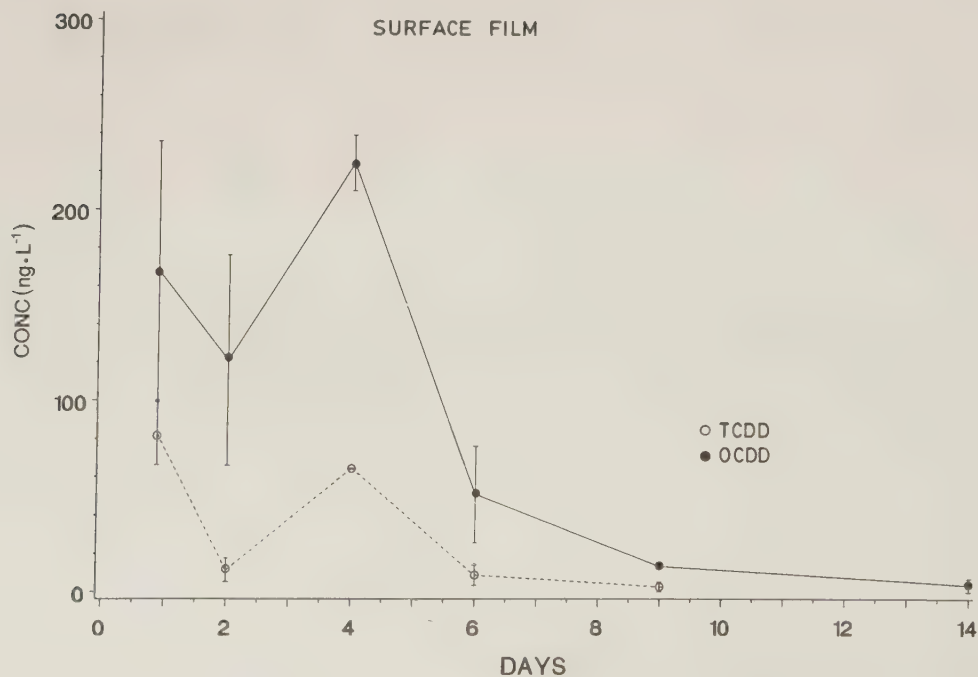


FIG. 2. Concentration of PCDDs (total ^{14}C) in surface films in lake enclosures. Bars represent the range in the enclosure means.

concentrations in periphyton declined slowly with a half-life of $193 \pm 32 \text{ d}$ ($R^2 = 0.62$). The concentrations of T_4CDD and O_8CDD in the colonized polyethylene were up to 6400 and 65 times, respectively, the concentrations in the periphyton (Fig. 5). The concentration (nanograms per square centimetre) of T_4CDD in the uncolonized polyethylene (i.e. placed in enclosures on day 0) was considerably higher than that from

the colonized polyethylene, but this trend was less obvious for O_8CDD .

The mean concentrations of O_8CDD and T_4CDD in sediments from jars were relatively constant over the 25-d sampling period (Fig. 6). However, the concentrations measured in sediments from jars were approximately 4 times higher than those from cores (Fig. 7). The sediment concentrations increased

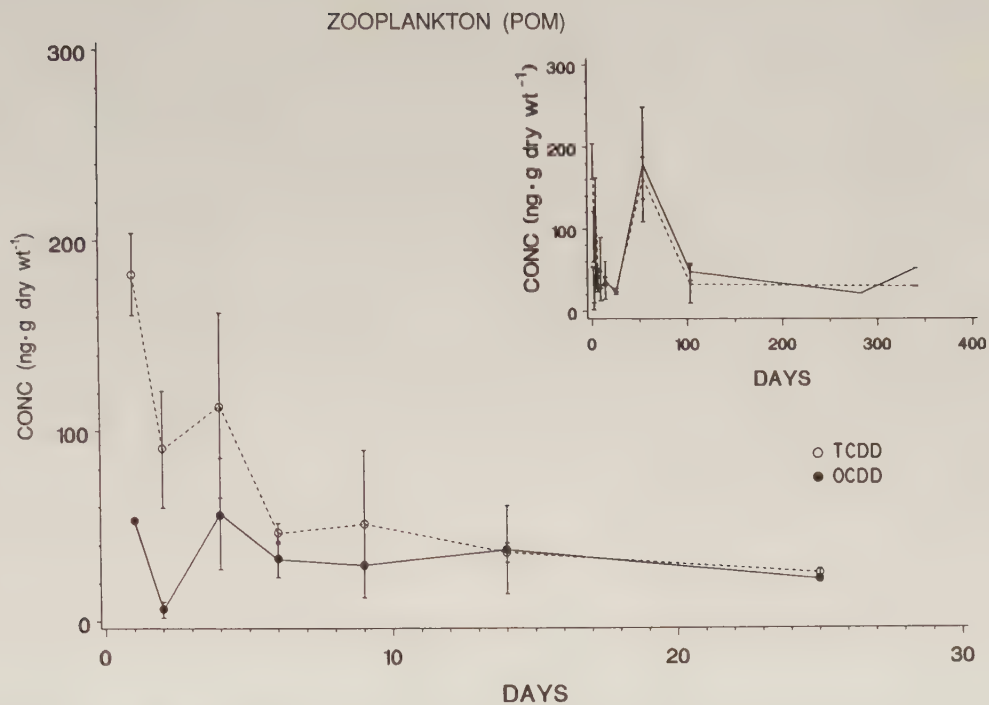


FIG. 3. Concentration of PCDDs (total ^{14}C) in POM $>100\ \mu\text{m}$ (zooplankton) in lake enclosures. Bars represent the range in the enclosure means.

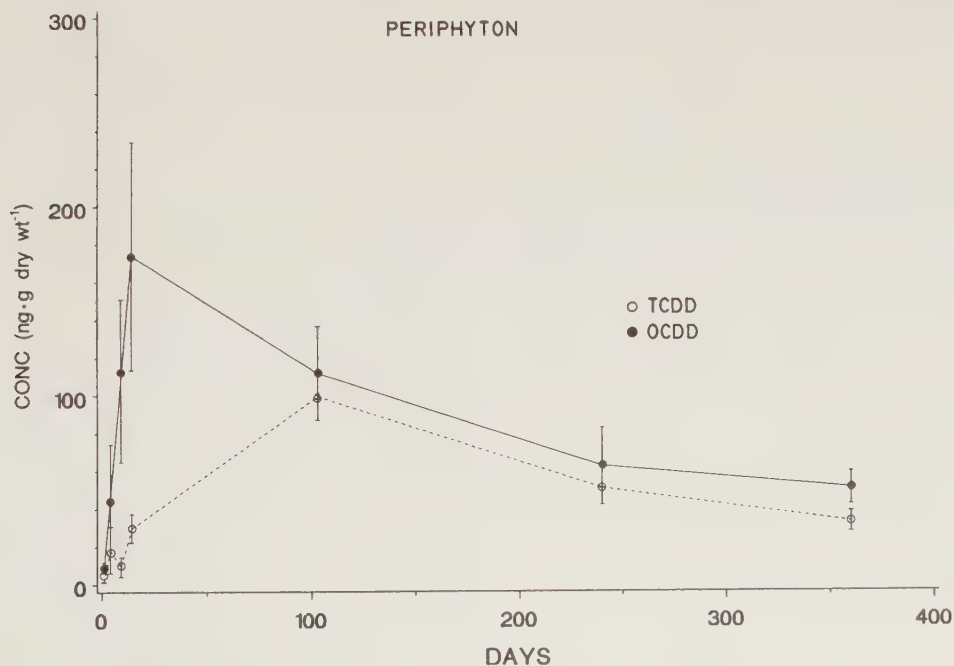


FIG. 4. Concentration of PCDDs (total ^{14}C) in periphyton in lake enclosures. Bars represent the range in the enclosure means.

slightly over the first few days and then remained relatively constant over 2 yr.

Analysis of sediment extracts by HPLC indicates that all ($>99\%$) of the T_4CDD remained as the parent compound even after 2 yr. Interpretation of the HPLC chromatograms for O_8CDD was difficult. All O_8CDD chromatograms showed a broad peak of activity between 3 and 5 min. This peak was eliminated if the sample was cleaned up using a silica Sep-Pak and eluted with 5 mL hexane, but recovery from the Sep-Pak was only 40%. Cleanup of the extracts using a C_{18} Sep-Pak and

eluting with 5 mL methanol resulted in a similar HPLC chromatogram. When this broad peak was collected, concentrated, and reinjected into the HPLC, it had the same retention time, i.e. 3–5 min. Shaking the extracts with concentrated sulfuric acid reduced the percentage of ^{14}C in the broad peak but did not eliminate it. When this peak was collected and analyzed by capillary gas chromatography (GC-ECD), an O_8CDD peak was not detected. However, if this broad peak was collected, shaken with concentrated sulfuric acid, and then passed through a normal phase Sep-Pak and eluted with hexane, an O_8CDD peak

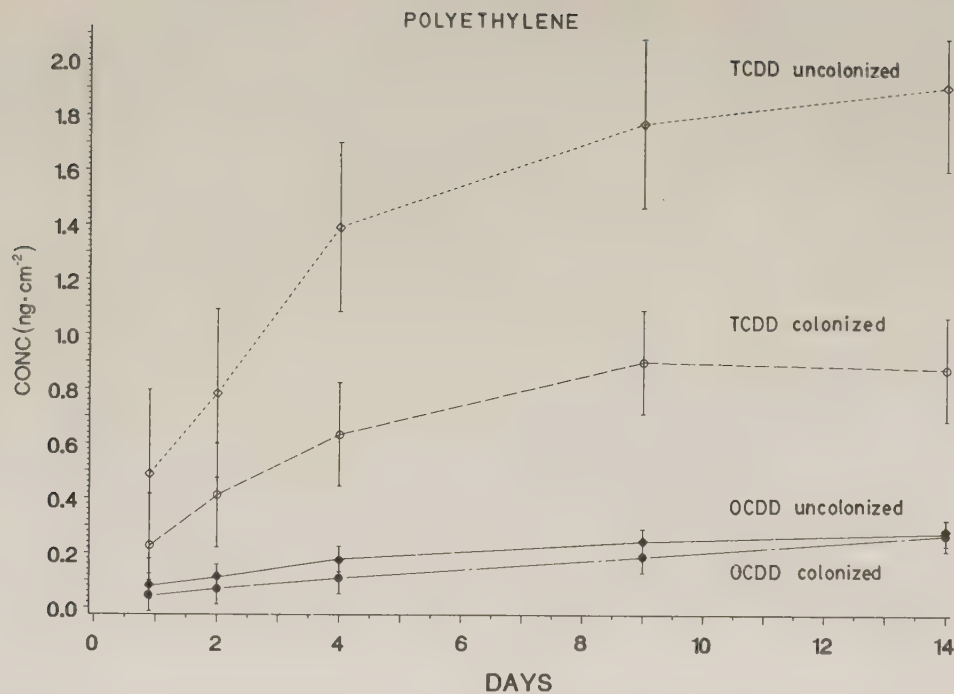


FIG. 5. Concentration of PCDDs (total ^{14}C) in polyethylene wall material in lake enclosures. Bars represent the range in the enclosure means.

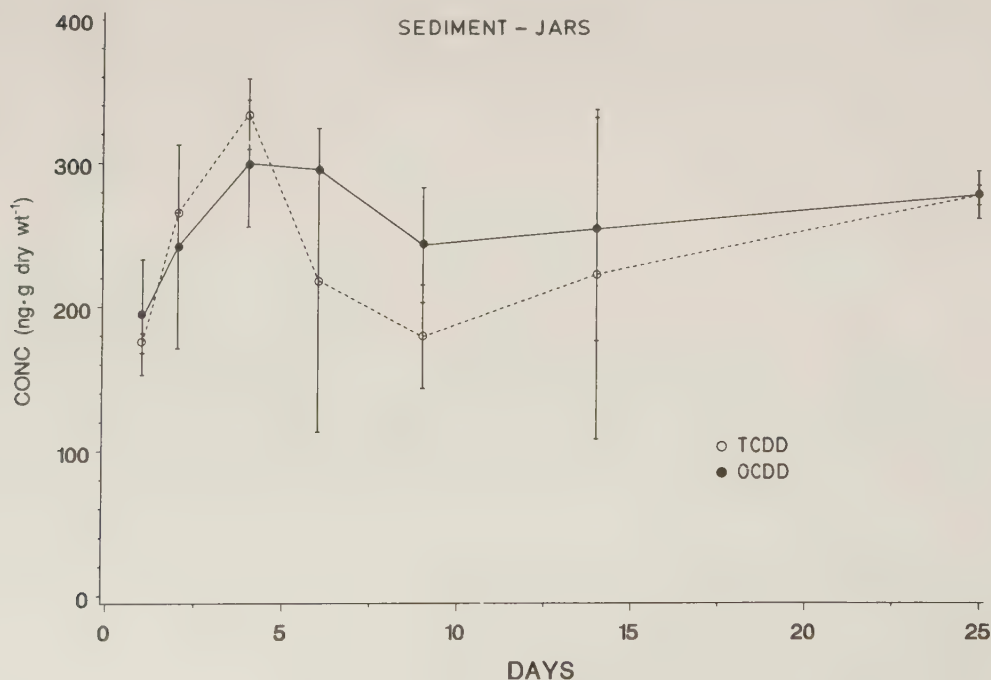


FIG. 6. Concentration of PCDDs (total ^{14}C) in sediment collected using jars in lake enclosures. Bars represent the range in the enclosure means.

was detected by GC-ECD. When a sediment extract from the reference enclosure was spiked with O_8CDD and then chromatographed by HPLC, it gave a similar broad peak. This indicates that the broad peak of activity was probably due to O_8CDD interacting with other components in the sample to change its HPLC chromatographic characteristics.

The $^3\text{H}_2\text{O}$ moved quickly into the interstitial waters reaching at least 10 cm within 3 d (Fig. 8). The ^3H activity was only slightly lower in the first 2 cm of sediment than in the overlying

water column. The $^3\text{H}_2\text{O}$ continued to penetrate into the sediments reaching at least 24 cm by day 54. The ^{14}C moved into the sediments more slowly than the $^3\text{H}_2\text{O}$. By day 3, the ^{14}C had not moved below 2 cm in any of the enclosures, and by day 8, only a small amount was detected in the 2–4 cm slice. By day 25, the ^{14}C had penetrated down to a depth of 6 cm. The subsurface peak in $[\text{}^{14}\text{C}]\text{O}_8\text{CDD}$ observed on day 25 was not observed in the replicate core on that date or in cores collected at later dates.

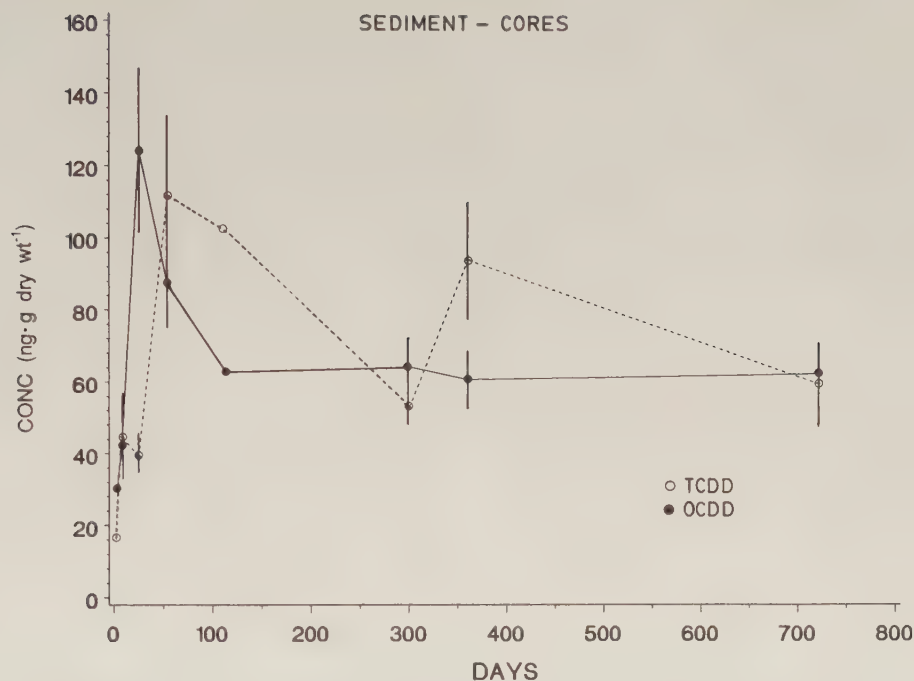


FIG. 7. Concentration of PCDDs (total ¹⁴C) in sediment collected using cores (0–6 cm) in lake enclosures. Bars represent the range in the enclosure means.

Discussion

Both PCDD congeners quickly partitioned/settled from the water column to the surficial sediments (Fig. 1, 7). Rapid movement of the PCDDs out of the water column was expected because of the mode of addition as a sediment slurry. The method of introducing the PCDDs to the water column is critical to the interpretation of the results. Friesen (1988) demonstrated that the method of addition (sediment slurry or solvent sprayover) of 1,2,3,4,7-P₅CDD to small ponds had a significant affect on its environmental fate, especially during the first few hours after the chemical additions. PCDDs were added to sediments containing a high percentage of organic carbon prior to being introduced into the enclosures. The PCDDs were strongly associated with the sediment particles which rapidly settled out of the water column.

T₄CDD is less hydrophobic than O₈CDD ($\log K_{ow} = 7.1$ and 8.2, respectively; Shiu et al. 1988), yet it was removed from the water column faster, with a half-life of 2.6 ± 0.2 d compared with 4.0 ± 0.3 d for O₈CDD. A similar trend was seen in pond studies where T₄CDD and O₈CDD had half-lives in the water column of 0.6–1.2 and 1.6–1.9 d, respectively (Corbet et al. 1988; Marcheterre et al. 1985). The initial concentrations of T₄CDD in unfiltered water were higher than those for O₈CDD as would be predicted by its higher water solubility (Friesen et al. 1985). However, the total concentrations of O₈CDD in the water column after 3 d remained higher than those of T₄CDD even though the water solubility of O₈CDD is more than 3 orders of magnitude lower. This inconsistency may be explained by the sorption of PCDDs to DOM or fine POM which may have a longer residence time in the water column. DOM has been previously shown to enhance the apparent water solubility of PCDDs (Webster et al. 1986; Servos and Muir 1989a). In this study, O₈CDD was almost totally bound to DOM or POM in the water column, while 10–15% of T₄CDD was truly dissolved. This difference in the partitioning of PCDDs between POM, DOM, and that in true solution is important in

interpreting the bioavailability and fate in the various compartments of the environment (Fig. 9).

Although no degradation products of [¹⁴C]T₄CDD were detected in the water column (1–5 d) or the sediments (1–720 d) and there is evidence to indicate that [¹⁴C]O₈CDD remained intact throughout the duration of the experiment, caution should be used when interpreting total ¹⁴C values. For example, even small amounts of more polar degradation products in the water could lead to overestimates of the actual amount of PCDDs passing through the reverse-phase cartridges, therefore overestimating the truly dissolved concentrations. This problem is most acute for the more hydrophobic O₈CDD congener. Although this does not appear to be the case, it is an alternative which cannot be ignored.

Both PCDD congeners had elevated concentrations (extractable ¹⁴C) in the surface microlayer (Fig. 2). The concentrations of the more hydrophobic O₈CDD were 2–20 times higher than those for T₄CDD. O₈CDD may preferentially partition into the hydrophobic environment of the surface microlayer (Meyers and Kawka 1982) or be incorporated into these films as the organic matter spiked into the enclosures (i.e. from the sediment) is incorporated into the surface films. Enrichment of surface films with hydrophobic organic contaminants relative to the water column has been reported in both marine and freshwater systems (Rice et al. 1982; Hardy and Kiesser 1988). The high concentrations of PCDDs found at the air–water interface in this study may have implications for the prediction of photolysis of these compounds in lake environments. The PCDDs in true solution may be more available for direct photolysis, although PCDDs may also undergo photosensitized transformations in the sorbed state (Zafiriou et al. 1984; Zepp et al. 1985; Miller et al. 1987).

The higher concentrations of T₄CDD (total ¹⁴C) in POM relative to O₈CDD during the first 6 d might have been due to the higher concentrations of PCDDs truly dissolved in the water column which resulted in more rapid uptake of T₄CDD by zooplankton (Fig. 3). Muir et al. (1985a) have shown that the

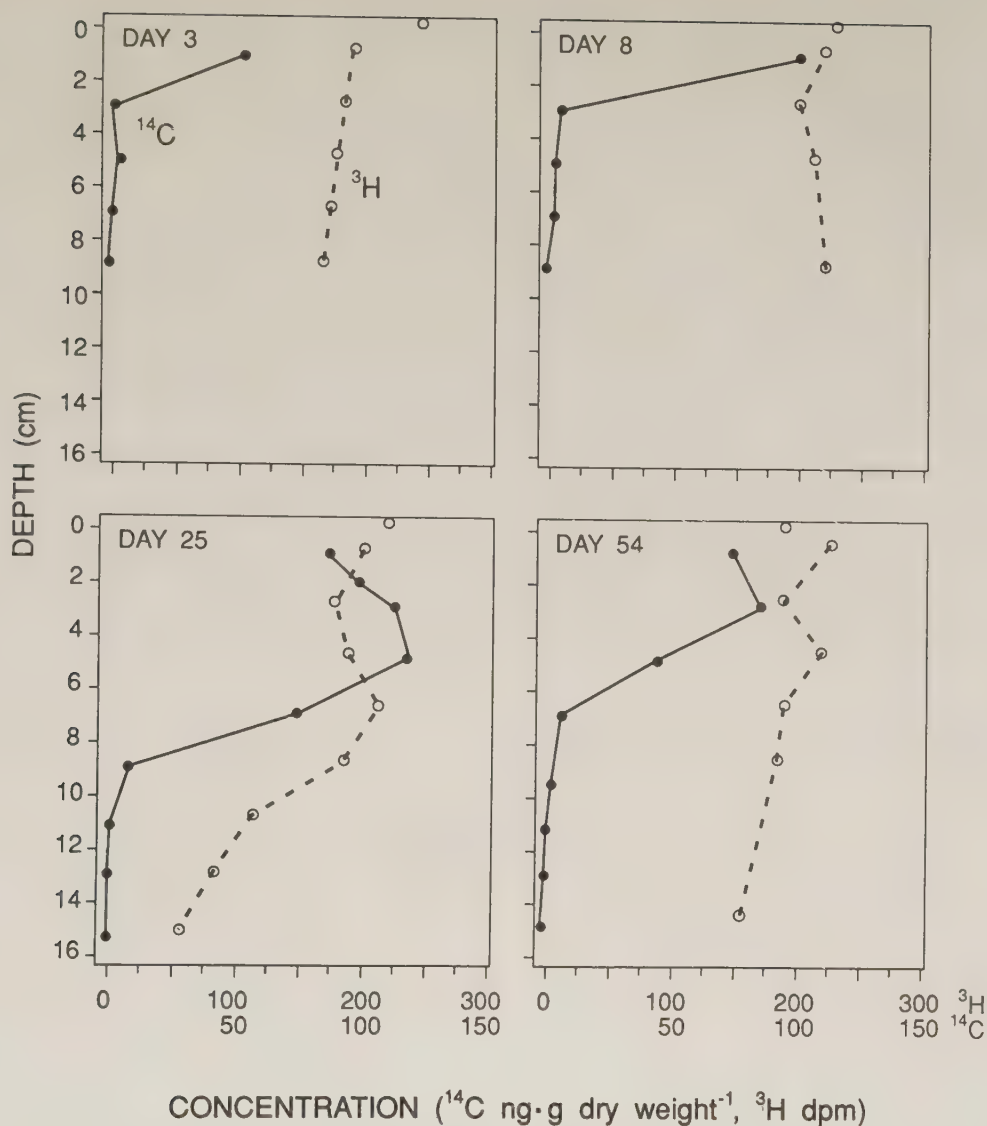


FIG. 8. Profile of $[^{14}\text{C}]\text{O}_8\text{CDD}$ and $^3\text{H}_2\text{O}$ in sediment cores from lake enclosures.

apparent uptake rate constants for PCDDs, especially those for the more hydrophobic congeners (e.g. O_8CDD), are considerably lower than those predicted from their K_{ow} alone. In laboratory studies, sorption of PCDDs to POM and DOM reduced the apparent uptake rate constants (and therefore bioconcentration factors (BCFs)) in fish and invertebrates by reducing the amount of PCDD in true solution and therefore bioavailable (Muir et al. 1985a; Servos and Muir 1989a). Although sorption to organic matter may reduce bioavailability, other factors such as steric hindrance may also be important (Oppenhuizen et al. 1985; Gobas and Mackay 1987; Gobas et al. 1988). The concentrations of T_4CDD in POM (zooplankton) declined rapidly from 160 to 205 $\text{ng}\cdot\text{g}^{-1}$ but remained above the detection limits at approximately the same concentrations as O_8CDD (20–67 $\text{ng}\cdot\text{g}^{-1}$). The low levels of PCDDs in net hauls may be due to suspended sediments in the water column resulting from the addition of the “spike” as a sediment slurry or resuspension of bottom sediments during sampling of sediments or biota. On day 54, all of the enclosures had a similar large increase in concentration to 108–248 $\text{ng}\cdot\text{g}^{-1}$. On this date, there was a threefold increase in the suspended carbon concentration

detected in the water column of the enclosures relative to earlier in the experiment and the lake site (Servos 1988). There was a sixfold increase in the sedimentation rate in Lake 304 open water during this same period (J. Curtis, Freshwater Institute, unpubl. data). This large increase may therefore be the result of a resuspension of sediments from wind action which affected the whole lake, including the enclosures, rather than the result of disturbing the bottom sediments during sampling.

Although O_8CDD is more hydrophobic than T_4CDD , it was present at lower concentrations (total ^{14}C) in the polyethylene wall material (Fig. 5). The sorption of O_8CDD to POM and DOM makes it less available to the polyethylene, even though the total O_8CDD concentrations in water were consistently higher than T_4CDD after day 3. A possible explanation for this may be that when the polyethylene is colonized by periphyton (3 wk), a more hydrophilic environment is created through which the PCDDs must diffuse before partitioning into the polyethylene. The concentration of T_4CDD in the colonized wall material was less than half of that in the noncolonized material on day 14. However, colonization of the wall material did not affect the concentration of O_8CDD in this compartment. The periphyton that could be scraped off of the polyethylene contributed less than 6% of the O_8CDD activity on the colonized wall material.

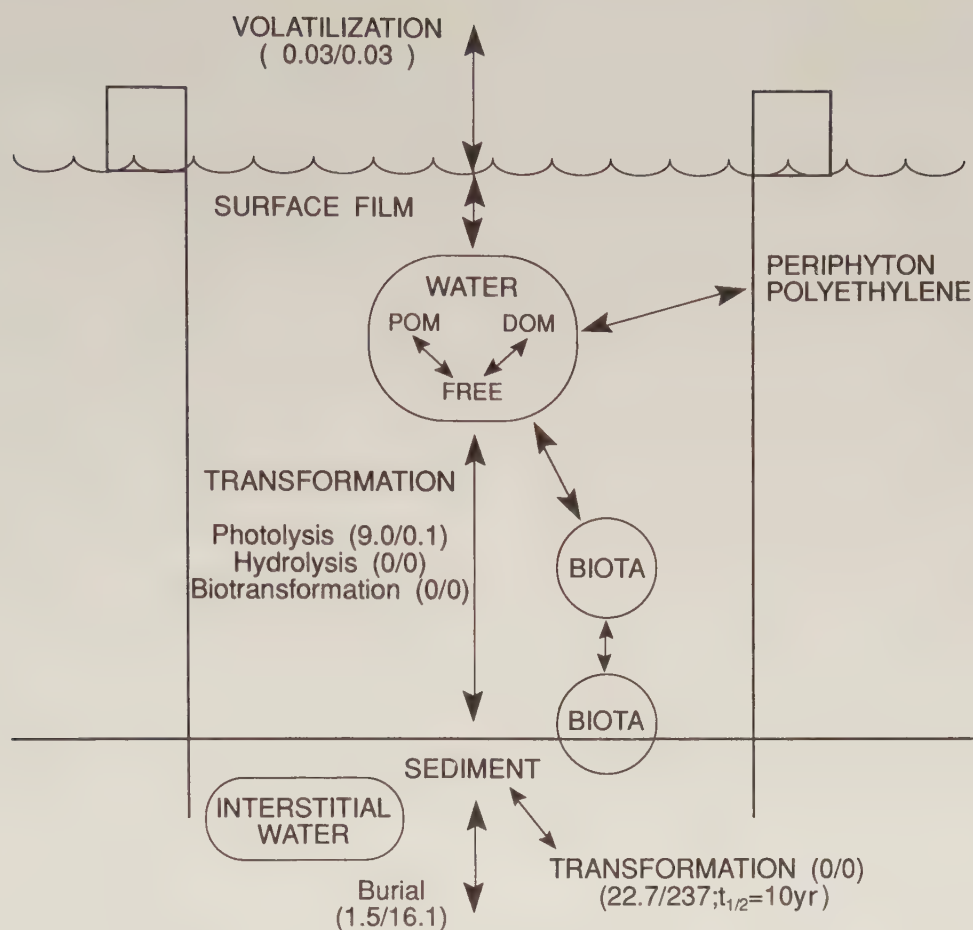


FIG. 9. Schematic representation of the environmental compartments and reactions in lake enclosures. Dominant process rates from the QWASI model are included in parentheses as $\text{mol} \cdot \text{h}^{-1} \cdot \text{Pa}^{-1}$ ($\text{T}_4\text{CDD}/\text{O}_8\text{CDD}$).

The high concentrations of O_8CDD relative to those of T_4CDD in periphyton seem inconsistent with other results (i.e. polyethylene). It is possible that the concentrations of PCDDs in periphyton were due primarily to a physical phenomenon rather than to direct partitioning. The concentrations of O_8CDD were not increased above those of T_4CDD until after day 4 when water concentrations had declined below $15 \text{ ng} \cdot \text{L}^{-1}$. After day 2, the total concentrations of O_8CDD in the water were higher than those of T_4CDD and were primarily associated with POM. The periphyton may have filtered out and trapped particles from the water column. Since the concentrations of O_8CDD associated with particles were greater than those of T_4CDD , this might have led to the higher concentrations of O_8CDD in periphyton. As total water concentrations declined with time, the concentrations of the two congeners in the periphyton became more similar, but those for O_8CDD remained higher, as they did in the water column.

Both congeners moved rapidly into the sediment (Fig. 6, 7). Sediment jar samples indicated that the sediment concentrations of T_4CDD and O_8CDD were similar over the first 24 d with both congeners reaching a maximum concentration of approximately $300 \text{ ng} \cdot \text{g dry weight}^{-1}$ by day 4 (Fig. 6). However, sediment core samples indicated that the sediment concentrations of both congeners peaked at approximately $120 \text{ ng} \cdot \text{g dry weight}^{-1}$ by 24–54 d (Fig. 7). If sediment concentrations derived from jars are used to determine a mass balance, then sediments would contain as much as 4 times the amount of ^{14}C actually added to the enclosures. The use of jars therefore appears to inflate the values for the sediment concentrations. The shape of the jars may result in overtrapping or focusing of the suspended material (^{14}C) added to the

enclosures. Sediment traps have been shown to collect an excess of material, especially under turbulent conditions (Hargrave and Burns 1979). This problem may have been exaggerated in this study because the jars were placed at the sediment–water interface and the PCDDs were added to the enclosures as a sediment slurry and mixed into the water column. Great care should therefore be used when interpreting any sediment data derived in this way (i.e. with jars). Burial or movement of PCDDs into the sediments ($>6 \text{ cm}$) was only minor during the first year of the experiment. $^3\text{H}_2\text{O}$ moved very rapidly into sediments below 10 cm by day 3, while the ^{14}C (most likely the intact congener) remained in the surface sediments (Fig. 8). Even after 54 d, very little of the ^{14}C had moved $>6 \text{ cm}$ into the sediment, even though the $^3\text{H}_2\text{O}$ had penetrated down to at least 24 cm. The rapid movement of $^3\text{H}_2\text{O}$ into the sediments may be at least partly due to an artifact caused by the physical structure of the enclosures. Heavy rainfall during the experiment may have created a head pressure which may have pressed the water through the sediments. However, the PCDDs apparently partitioned strongly to the highly organic (25.2% total carbon) sediments and remained at the surface. With time, the PCDDs (total ^{14}C) slowly penetrated into the sediments either by moving with the interstitial waters (free or sorbed to the dissolved organic matter) or by turbulent or biological mixing of contaminated sediment particles (Anderson et al. 1987). The “apparent” K_p of T_4CDD was experimentally determined using Lake 304 sediments to be $<10 \text{ L} \cdot \text{kg}^{-1}$ at very high suspended sediment concentrations (i.e. $>10^4 \text{ mg} \cdot \text{L}^{-1}$; Servos and Muir 1989b). Assuming a concentration in sediment of $120 \text{ ng} \cdot \text{g dry weight}^{-1}$ and a K_p of 10 the estimated total

concentration of T₄CDD in sediment interstitial water would be approximately 0.5 ng·L⁻¹. Although no ¹⁴C could be detected (<0.5 ng·L⁻¹) in the interstitial waters (28.8 ± 3.6 mg DOC·L⁻¹), this does not preclude DOM as a means of transport of PCDDs into the sediments. DOM from Lake 304 interstitial waters has been shown to inflate the apparent solubility of T₄CDD (Servos and Muir 1989a). Sorption of PCDDs to DOM may increase their mobility in sediments.

Sediment core samples indicate little or no decrease in the sediment concentrations (i.e. total ¹⁴C) of either congener over 2 yr (Fig. 8). The sediment core data did not fit the first-order kinetic rate model (*t* > 25 d), i.e. the slope was not significantly different from zero. This may be due in part to the large variability among samples within each enclosure. No degradation products of [¹⁴C]T₄CDD were detected using HPLC over the duration of the study. [¹⁴C]O₈CDD likely also remained intact in the sediment, although this was not confirmed.

Corbet et al. (1988) showed that 1,3,6,8-T₄CDD in sterile and non-sterile pond water degraded at a similar rate (half-life = 6.3–8.0 d) in sunlight but did not degrade under darkened conditions. Photolysis may have been an important pathway for removal of the T₄CDD from the enclosures, even though no degradation products were detected in the water column during the first 5 d or in the sediments for duration of the experiments. The QWASI model predicts direct photolysis to be an important degradative pathway for both T₄CDD and O₈CDD (Table 2). Corbet et al. (1988) showed that T₄CDD had a half-life of 6.3 d when exposed to sunlight in autoclaved pond water and concluded that photolysis was a major route of degradation in ponds. Although photolysis may be an important route of removal of PCDDs from the lake enclosures the actual rates of photolysis may only be significant during the first few days of the experiment when the concentrations truly dissolved are relatively high.

Corbet et al. (1988) reported two polar degradation products of T₄CDD in sediment of small ponds by day 54 at 6.3 and 16% of the total ¹⁴C. This difference may be due to several factors including the smaller depth (<1 m) and size (5 m³) and lower organic carbon content of the sediment (3.5%) of the ponds relative to the lake enclosures. The low organic content of the pond sediments might have resulted in a slower movement from the water to the sediment compartment, therefore increasing the availability of T₄CDD for biological and chemical interactions. T₄CDD in these pond studies was added with acetone as a cosolvent at much higher nominal concentrations (viz. 980, 240, 98 ng·L⁻¹). Because T₄CDD in the present study was adsorbed to the sediments prior to being added to the enclosures, the amount truly dissolved and therefore available to undergo direct photolysis or biotransformation was reduced. Quensen and Matsumura

(1983) found that 2,3,7,8-T₄CDD in laboratory experiments was transformed only during the initial phases of the experiments. They suggested that this was due to microorganisms which utilized the initially high concentration of truly dissolved 2,3,7,8-T₄CDD. This same trend was observed for 1,3,6,8-T₄CDD in sediment/water incubations (Muir et al. 1985b). Muir et al. (1985b) reported a maximum of 7% of the 1,3,6,8-T₄CDD in these laboratory systems as polar metabolites over 675 d. This low rate of transformation in water/sediment systems observed in the laboratory is supported by these enclosure studies. A large proportion (94–104%) of both PCDDs could be accounted for as untransformed compound in the sediment on days 24–54 (Table 3). This suggests low rates of transformation and removal from the system.

A large proportion of both congeners (as total ¹⁴C) remained in the sediments (55–81%) T₄CDD and 57–58% O₈CDD after 1 yr. Corbet et al. (1988) reported less than 14.2% of the T₄CDD remaining in small ponds after 426 d, and Tsushimoto et al. (1982) reported only 29.4% of the 2,3,7,8-T₄CDD remaining in slightly larger ponds after 25 mo. A larger fraction (approximately 7.2 times given log *K_p* = 6.53) of the T₄CDD in the less organic pond sediments would be expected to be in true solution and therefore available for volatilization, photolysis, etc. Although the truly dissolved concentrations would be very low, over 2 yr, this slight difference may be significant. In contrast with the results for T₄CDD, Marcheterre (1985) reported 61.8–88.2% of the O₈CDD remaining in small ponds (same site as Corbet et al. 1988) after 652 d. The higher retention of O₈CDD is expected because of its reduced availability in the water column. The fact that similar amounts of T₄CDD and O₈CDD could be accounted for in the lake enclosures may be due again to the high organic content of the lake sediments.

PCDDs are generally considered to be extremely persistent in sediment, with half-lives >10 yr (Miller and Zepp 1987; Muir et al. 1985b). The rates of microbial transformation in the sediment are therefore usually considered to be zero. Because most of the PCDDs are associated with the sediments, the predictions of the QWASI model are sensitive even to small rates of transformation out of the sediment. If the half-life of the PCDDs in sediment is assumed to be 10 yr, transformation in the sediment is predicted to be the dominant degradative pathway. Burial in the sediments may be the dominant process removing PCDDs, especially the more hydrophobic O₈CDD, from the aquatic system. Using a sedimentation rate of 0.2–1.0 g·m⁻²·d⁻¹ (Lake 304 open water, J. Curtis, Freshwater Institute, unpubl. data), PCDDs could be buried below 6 cm within 4–20 yr. The QWASI model predicts burial of PCDDs in the sediments to be relatively important compared with removal by transformation or volatilization.

TABLE 3. Mass balance of ¹⁴C in aquatic mesocosms^a (mean ± range). nd = not detectable.

Time	Percentage accounted for									
	O ₈ CDD					T ₄ CDD				
	Water	Surface film	Peri/plastic	Sediment	Total	Water	Surface film	Peri/plastic	Sediment	Total
2 h	30 ± 2	—	—	—	—	64 ± 2	—	—	—	—
2–3 d	41 ± 6	1	1	32	76	41 ± 1	<1	6 ± 1	21	70
8–9 d	—	<1	3	42 ± 6	—	—	nd	14 ± 1	43 ± 6	—
24 d	2	nd	—	104 ± 18	106	2	nd	—	39 ± 4	41
54 d	1	—	—	76 ± 10	78	<1	—	—	94 ± 17	95
103 d	<1	—	—	56	56	<1	—	—	81	81
271 d	<1	—	—	58 ± 5	58	<1	—	—	50 ± 6	50
360 d	<1	—	—	56 ± 5	58	<1	—	—	81 ± 4	81
720 d	<1	—	—	57 ± 4	57	<1	—	—	55 ± 9	55

^aCorrected for extraction efficiency and assuming 67 m³ of water, 20-m² surface area, 39 m² of polyethylene, and 30 kg dry weight of sediment (6 cm).

Astle et al. (1987) used a similar fugacity-based model to predict the environmental dynamics of PCDDs in Lake Siskiwit and concluded that reaction rates would be small relative to the transport rates (sedimentation) of PCDDs to the sediment. Astle et al. (1987) also predicted that 14% of the tetra-CDDs and <1% of the O₈CDD in the water column would be truly dissolved in solution and therefore available for biological uptake or chemical reaction. These results indicate that future work should focus on determining the factors controlling the removal of PCDDs from the system by sedimentation (e.g. K_p) and eventual burial in sediments.

Although enrichment of the more highly chlorinated PCDDs in lake sediments may be due to higher inputs, selective retention of these congeners in aquatic systems may be a contributing factor. The lower availability of the higher chlorinated PCDDs for chemical or biological transformation in the water column and the rapid removal (sedimentation) to the sediments may lead to relatively greater persistence than of lower chlorinated PCDDs in aquatic systems. However, the observation that both congeners in the lake enclosures were retained in similar amounts indicates that the movement of the particle-associated PCDDs is so rapid that surficial sediments directly reflect inputs into the system. The selective retention of higher chlorinated congeners combined with the higher atmospheric inputs of these congeners may explain the pattern of increasing concentrations of PCDD congeners in sediment with increasing chlorine substitution observed in the Great Lakes and other aquatic environments.

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Bioavailability of Polychlorinated Dibenzo-*p*-dioxins in Lake Enclosures

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The bioavailability of 1,3,6,8-tetra- (T_4 CDD) and octachlorodibenzo-*p*-dioxin (O_8 CDD) was examined in large (40 m³) lake enclosures at the Experimental Lakes Area in northwestern Ontario. The polychlorinated dioxins (PCDDs) were added to replicate enclosures as a sediment slurry at a nominal concentration of 58–59 ng·L⁻¹. T_4 CDD was more bioavailable to caged benthic invertebrates and fish (white sucker, *Catostomus commersoni*) than O_8 CDD immediately after the addition to the enclosures. However, as the concentration of T_4 CDD in the water column rapidly declined, the bioavailability of T_4 CDD also declined. Sorption of PCDD to organic matter and rapid partitioning to sediments might have reduced the uptake of PCDDs directly from the water column. Accumulation of PCDDs in biota appeared to shift from direct equilibrium partitioning during the first few days, when the concentrations in the water column were relatively high, to a detrital food chain transfer as the freely available PCDDs in the water declined. This conclusion is supported by the results of the simple, four-compartment food chain model of Thomann and Connolly based on the uptake kinetics of PCDDs from water and food.

La biodisponibilité de la 1,3,6,8-tétra- (T_4 CDD) et de l'octachlorodibenzo-*p*-dioxine (O_8 CDD) a été étudiée dans de grandes enceintes (40 m³) dans la région des lacs expérimentaux du nord-ouest de l'Ontario. On a ajouté les dioxines polychlorées (PCDD) dans plusieurs enceintes identiques, sous forme de boue de sédiments, à une concentration nominale de 58–59 ng·L⁻¹. La T_4 CDD était plus biodisponible pour les invertébrés benthiques en cage et les poissons (meunier noir, *Catostomus commersoni*) que l' O_8 CDD immédiatement après l'addition dans les enceintes. Toutefois, alors que la concentration de T_4 CDD dans la colonne d'eau diminuait rapidement, la biodisponibilité de la T_4 CDD diminuait également. La sorption des PCDD à la matière organique et la séparation rapide des sédiments peuvent avoir diminué l'absorption directe des PCDD à partir de la colonne d'eau. L'accumulation des PCDD dans le biote semblait passer de la séparation directe à l'équilibre (observée au cours des premiers jours, alors que les concentrations dans la colonne étaient relativement élevées) à un transfert à la chaîne alimentaire détritique, à mesure que la concentration des PCDD disponible en solution dans l'eau diminuait. Cette conclusion est appuyée par les résultats du modèle simple de chaîne alimentaire à quatre compartiments de Thomann et Connolly, basé sur la cinétique de l'absorption des PCDD à partir de l'eau et des substances nutritives.

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Polychlorinated dibenzo-*p*-dioxins (PCDDs) are extremely hydrophobic compounds which partition rapidly to organic matter in aquatic environments (Servos et al. 1992). The concentration of PCDD congeners in sediments in most aquatic environments generally increases with increasing chlorine substitution (Czuczwa and Hites 1986; Ballschmiter et al. 1986; Hagenmaier et al. 1986). This is likely caused by larger inputs and greater persistence of the higher chlorinated congeners (Czuczwa and Hites 1986; Servos et al. 1992). However, this pattern is not directly reflected in the concentration of PCDD congeners found in aquatic biota (De Vault et al. 1989; Rappe et al. 1987; Miyata et al. 1987). There is no consistent pattern in the concentration of PCDD congeners found in biota except that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8- T_4 CDD) is often the dominant congener (Rappe et al.

1987; Kuehl et al. 1987b). Other congeners, including octachlorodioxin (O_8 CDD), are also commonly found in aquatic biota (Miyata et al. 1987; Clement et al. 1987; Kuehl et al. 1987a). A simple extrapolation of the relationship between the octanol–water partition coefficient (K_{ow}) and bioconcentration factor (BCF) described by Veith et al. (1979) predicts extremely high BCFs (10^5 – 10^6) for the higher chlorinated PCDDs. However, several authors (Muir et al. 1985a; Bruggeman et al. 1984; Gobas and Mackay 1987; Servos et al. 1989) have reported a decline in the BCFs of PCDDs and other compounds with log K_{ow} greater than approximately 6.5. The extremely low concentrations of PCDDs in true solution and the relatively high concentrations in the sediments, especially of the more highly chlorinated PCDDs, may result in a detrital food chain transfer rather than direct equilibrium partitioning from the water column.

The objective of this study was to examine the bioavailability of two PCDDs representing the extremes in the physical chemical properties of the environmentally significant PCDDs,

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TABLE 1. Mean concentration ($\pm$ SD) of PCDDs in caged benthic invertebrates from lake enclosures. nd = not detectable.

	T ₄ CDD (ng·g ⁻¹)	N	O ₈ CDD (ng·g ⁻¹)	N	Detection limit
Day 10 (0–10)					
<i>Hyalella azteca</i>					
Water	22.9 $\pm$ 8.9	12	3.2 $\pm$ 1.7	10	2.0
Sediment	11.1 $\pm$ 4.2	13	nd	9	2.0
<i>Limnephilus</i> sp.	14.3 $\pm$ 3.9	9	5.9 $\pm$ 2.5	8	1.5
<i>Ephemerella</i> sp.	20.3 $\pm$ 5.4	6	nd	6	3.0
<i>Amnicola limosa</i>	19.0 $\pm$ 3.5	6	2.8 $\pm$ 0.5	6	1.1
<i>Crangonyx laurentianus</i>	18.5 $\pm$ 2.8	11	nd	11	0.6
Day 24 (14–24)					
<i>Hyalella azteca</i>					
Water	nd	5	nd	5	2.0
Sediment	nd	5	nd	5	2.0
<i>Limnephilus</i> sp.	4.3 $\pm$ 1.7	8	4.8 $\pm$ 1.1	8	1.5
<i>Ephemerella</i> sp.	Trace	4	nd	4	3.0
<i>Amnicola limosa</i>	3.9 $\pm$ 0.6	2	nd	2	1.1
Day 371 (361–371)					
<i>Crangonyx laurentianus</i>	6.4 $\pm$ 3.8	36	nd	36	2.5

TABLE 2. Mean concentration ($\pm$ SD) of PCDDs in unionid clams held in cages in lake enclosures.

Time	T ₄ CDD (ng·g ⁻¹)		O ₈ CDD (ng·g ⁻¹)	
	Water	Sediment	Water	Sediment
10 d	25.2 $\pm$ 10.5	21.4 $\pm$ 6.0	2.8 $\pm$ 1.3	2.9 $\pm$ 1.2
24 d	1.6 $\pm$ 0.5	0.9 $\pm$ 0.5	0.7 $\pm$ 0.6	0.5 $\pm$ 0.4

under natural field conditions simulating lakes. The bioavailability of 1,3,6,8-tetrachloro- (T₄CDD) and octachlorodibenzo-*p*-dioxin (O₈CDD) were studied in large lake enclosures at the Experimental Lakes Area in northwestern Ontario. The environmental fate of these congeners in these lake enclosures has been reported by Servos et al. (1992). The concentrations of the two isomers in caged benthic invertebrates and fish held in the water column or on the sediments were compared. A compartmental model developed by Thomann and Connolly (1984) was used to predict the relative importance of direct partitioning from water versus detrital food chain transfer in explaining the concentration of PCDDs found in biota.

Methods

Five 5 m diameter $\times$ 2 m deep (40 m³) enclosures were constructed in the littoral zone of Lake 304 in the Experimental Lakes Area in northwestern Ontario (Johnson and Vallentyne 1971). The construction and chemical characteristics of the enclosures are reported in more detail in Servos et al. (1992). On June 12, 1985, two enclosures were treated with [¹⁴C]T₄CDD (894 MBq·mmol⁻¹) and two with [¹⁴C]O₈CDD (761 MBq·mmol⁻¹) at a nominal concentration of 58–59 ng·L⁻¹. A fifth enclosure served as a control. PCDDs were added to the enclosures as a sediment slurry as described in Servos et al. (1992).

Benthic Invertebrates

Several species of benthic invertebrates were exposed in cages at the sediment–water interface for 10-d intervals several times during the experiment (viz. days 0–10, 14–24, and 94–

104). Cages were made from 500-mL Nalgene bottles with two thirds of the walls cut away and covered with 0.45-mm mesh. One species (*Hyalella azteca*) was also held in cages hung in the water column during the same time periods. Benthic invertebrates were collected from each bottle at the end of each exposure period and frozen. Benthic invertebrates were blotted dry with a paper towel, weighed, and lyophilized. One to four organisms were pooled and oxidized using a Packard 306 sample oxidizer (Packard Instruments Co., Downers Grove, IL). The ¹⁴C was trapped in 2-methoxyethylamine, diluted in scintillation fluor, and assayed by liquid scintillation counting (LSC) (LC 7500, Beckman Instruments, Irvine, CA).

Unionid Clams

Unionid clams (*Eliptio complanata*) were collected from Lake 377 (Experimental Lakes Area) and held for 2 wk before being placed in cages in the enclosures 1 h after the PCDD addition. In each enclosure, one cage was held on the sediment and a second cage was allowed to float at the surface. Cages were constructed with a wooden frame (0.6 $\times$ 0.6 $\times$ 0.9 m) covered 56% with 1-mm mesh and 44% with 5-mm mesh. Ten clams (16.4 $\pm$ 5.3 g soft tissue wet weight) were collected on day 10 or 24 as described above. The shell was removed from each clam and the soft tissues weighed and lyophilized. Each individual clam was homogenized in 50 mL of toluene using a Polytron. Samples were centrifuged at 2000g for 10 min. A 1-mL aliquot of the supernatant was diluted with scintillation fluor (Atomlight, New England Nuclear) and assayed by LSC. The unextractable residue was air-dried and a 0.2-g subsample combusted on a Packard sample oxidizer. Selected extracts were further assayed by high-performance liquid chromatography (HPLC) using a Waters model 6000A solvent delivery system and a μ Bondapak C₁₈ column (Waters Scientific, Milford, MA) to determine the actual level of ¹⁴C as parent T₄CDD and O₈CDD. The mobile phase was methanol (1.5 mL·min⁻¹), and 0.5-min fractions were collected and assayed by LSC. T₄CDD and O₈CDD had retention times of 4.3 and 6.2 min, respectively. Extracts were shaken with 1 mL of concentrated H₂SO₄ to remove lipids. The toluene was rotoevaporated under reduced pressure to <5 mL, further reduced to near dryness under a

stream of N₂, and finally made up to 0.5 mL in chloroform. BCFs were determined as

$$(1) \text{ BCF}_a = C_b/C_w$$

where BCF_a is the apparent bioconcentration factor, C_b is the concentration in biota (nanograms per kilogram) and C_w is the time-weighted mean water concentration (nanograms per litre). The predicted concentration in biota was determined by rearranging equation (1) and assuming a true BCF of 5017 for T₄CDD and 705 for O₈CDD. These BCFs represent the highest values reported in rainbow trout (*Oncorhynchus mykiss*) in synthetic water (Servos et al. 1989). Concentrations of T₄CDD and O₈CDD truly dissolved in water were determined as the time-weighted mean multiplied by the percentage in true solution determined during the first 48 h (Servos et al. 1992), i.e. 15% for T₄CDD and 1% for O₈CDD.

Fish

White suckers (*Catostomus commersoni*) collected from a nearby lake were held in Lake 304 for 48 h before being placed in the enclosures 1 h after the PCDD addition. Ten fish (12.5 ± 3.6 g wet weight) were held in each of the cages described above (i.e. one on the sediment and one at the surface) and sampled on days 10 and 24. Ten white suckers were also added directly into each enclosure and as many as possible retrieved on days 104–106 using small minnow traps. The gills and the digestive tract (from the esophagus to the rectum, including the contents) were removed from seven fish from each cage and each sample was (gill, digestive tract, carcass) weighed, lyophilized, and then reweighed separately. The carcass and gills were homogenized in toluene (15.0 or 4.1 mL, respectively) using a Polytron. Samples were centrifuged at 2000g for 10 min and a 2- to 5-mL aliquot of the supernatant was diluted with scintillation fluor and assayed by LSC. A single extraction removed greater than 90% of the extractable PCDDs. The extracted sample was air-dried and the entire sample (gills) or a 0.1-g subsample (carcass) combusted on a Packard sample oxidizer. The digestive tract samples were combusted directly without freeze-drying or extraction. Selected extracts were also assayed by HPLC as described above.

Results

Benthic Invertebrates

The concentration of T₄CDD in benthic invertebrates was higher (11.1–22.3 ng·g⁻¹) than O₈CDD (<5.9 ng·g⁻¹) on day 10 (Table 1). By day 24 the concentration of T₄CDD in benthic invertebrates had declined to levels similar to those for O₈CDD (i.e. <4.3 ng·g⁻¹). By day 371 the concentration of

T₄CDD in the one species examined was similar to those in other benthic invertebrates on day 24, while O₈CDD was not detectable. However, the detection limits were relatively high (0.6–3.0 ng·g⁻¹) because of the small sample weights. *Hyaella azteca* held in the water column had significantly higher concentrations of T₄CDD than those held on the sediments at the same time.

Unionid Clams

The unionid clams showed a trend similar to the benthic invertebrates in that T₄CDD was 20–30 ng·g⁻¹ on day 10 and declined to concentrations similar to those of O₈CDD by day 24 (i.e. 0.2–2.0 ng·g⁻¹) (Table 2). Clams exposed in cages held in the water column had slightly higher mean concentrations than those held on sediments on both days 10 and 24, but the results were not significantly different (ANOVA, $p > 0.05$). All of the ¹⁴C found in clams was extractable in toluene, and HPLC analysis indicated that >95% of the [¹⁴C]T₄CDD remained as the parent compound.

Fish

The relative concentration of PCDDs in caged fish showed a trend similar to the other biota with higher concentrations of T₄CDD than O₈CDD (Table 3). The concentrations of T₄CDD in both the gills and carcasses of fish were significantly higher than those of O₈CDD on day 10 but declined to concentrations similar to those of O₈CDD by day 24. The concentrations in the digestive tract differed such that T₄CDD was higher than O₈CDD on day 10 but similar by day 24. Concentrations in the digestive tract were approximately 10 times higher than in the other tissues on both dates. The concentrations of PCDDs in fish (carcass) held in the water column were slightly higher than those in fish held on the sediment, but the differences were not significant with one exception: the fish exposed in the water column had higher concentrations in the carcass on day 24. The concentrations of both T₄CDD and O₈CDD in the gill and carcass of fish which were released into the enclosures and retrieved on day 104 were relatively low (0.1–0.5 ng·g⁻¹) but similar (Table 4). The concentrations in the digestive tracts were still 2–10 times higher than those in the other tissues. On day 10, more than 50% of the [¹⁴C]T₄CDD in gills was extractable in toluene whereas on day 24, less than 25% was extractable (Table 3). Sixty-eight percent of the [¹⁴C]CDD in extracts analyzed by HPLC remained as the parent compound. The percentage of extractable [¹⁴C]T₄CDD in the carcass was slightly less than in the gills on day 10 and this percentage also decreased on day 24. [¹⁴C]O₈CDD also showed a similar pattern.

TABLE 3. Mean concentration (±SD) of PCDDs in white suckers held in cages in the water column (water) and on the sediment lake enclosures.

Time	Tissue	T ₄ CDD (ng·g ⁻¹)		O ₈ CDD (ng·g ⁻¹)	
		Water	Sediment	Water	Sediment
10 d	Gill	25.2 ± 16.6	21.4 ± 16.7	2.8 ± 1.4	2.9 ± 2.7
	Carcass	4.2 ± 3.3	3.1 ± 1.9	2.9 ± 2.4	1.1 ± 0.9
	Digestive tract	125.7 ± 53.5	133.0 ± 75.9	33.9 ± 22.4	68.7 ± 70.2
24 d	Gill	3.9 ± 2.4	2.1 ± 0.9	3.8 ± 2.4	1.3 ± 0.8
	Carcass	1.0 ± 0.5	0.5 ± 0.2	0.9 ± 0.3	0.4 ± 0.2
	Digestive tract	10.0 ± 3.7	16.3 ± 5.4	13.3 ± 6.7	12.0 ± 6.7

TABLE 4. Mean concentration ($\pm$ SD) of PCDDs in white suckers held in lake enclosures for 104–106 d.

Tissue	T ₄ CDD (ng·g ⁻¹) (n = 4)	O ₈ CDD (ng·g ⁻¹) (n = 6)	Detection limit
Gill	3.5 $\pm$ 2.0	2.8 $\pm$ 1.4	0.20
Carcass	1.8 $\pm$ 1.4	2.7 $\pm$ 1.8	0.01
Digestive tract	50.3 $\pm$ 38.1	13.9 $\pm$ 14.8	0.01

Discussion

The concentrations of T₄CDD in caged biota exposed in lake enclosures on days 0–10 were approximately 5 times higher than concentrations of O₈CDD (Table 1). The higher concentrations of T₄CDD compared with O₈CDD reflect the higher availability of T₄CDD in the enclosures. The percentage of the total concentration in true solution, determined using reverse-phase cartridges, was 10–15% for T₄CDD compared with less than 1% for O₈CDD (Servos et al. 1992). Although O₈CDD is more lipophilic (log K_{ow} = 8.2 for O₈CDD and 7.1 for T₄CDD), the strong affinity of O₈CDD for organic matter in the water column may limit its bioavailability relative to T₄CDD (Muir et al. 1985a). Hydrophobic compounds bound to particulate (POM) and dissolved organic matter (DOM) are generally not considered to be bioavailable (Landrum et al. 1987; McCarthy 1983; Servos et al. 1989; Servos and Muir 1989a).

The apparent BCFs of T₄CDD measured for biota in the lake enclosures on days 10 and 24 were 231–1598, which are similar to those reported for invertebrates (794–2846) and fish (1400–5840) in the laboratory (Muir et al. 1985b, 1986; Servos et al. 1989). Apparent BCFs for O₈CDD on day 10 were considerably lower (78–565) and also comparable with those reported from the laboratory for invertebrates (42–160) and fish (34–2226) (Muir et al. 1985b, 1986; Servos et al. 1989). Apparent BCFs in lake enclosures were also similar to those reported for T₄CDD in small ponds for snails (1860–4180) and for O₈CDD in small ponds for fish (38–1300) (Corbet 1983; Marcheterre 1985).

The concentrations of T₄CDD detected in biota on both days 10 and 24 were within the range predicted by its BCF measured in the laboratory (Table 5). The large decline in the concentration of T₄CDD in biota on day 24 relative to day 10 was likely due to the rapid decline in T₄CDD concentrations in the water (40.2–0.6 ng·L⁻¹) as T₄CDD partitioned into the other environmental compartments, primarily the sediment (Servos et al. 1992). In contrast, the concentration of O₈CDD in biota declined only slightly between days 10 and 24, and the concentration of O₈CDD in biota could not be accounted for by its BCF (Table 5). Concentrations of O₈CDD in biota were at least an order of magnitude greater than predicted by its laboratory BCF. In addition, because the fraction of O₈CDD in true solution was assumed to be 1% and the actual percentage

TABLE 5. BCFs and predicted concentrations for PCDDs in biota held in lake enclosures.

	T ₄ CDD		O ₈ CDD	
	Concentration (range)	BCF	Concentration (range)	BCF
Days 0–10				
Water (ng·L ⁻¹) ^a	13.4 (40.2–2.7)		14.0 (26.1–6.1)	
Biota (ng·g ⁻¹)				
Predicted ^b	10.1	5017	0.1	705
Invertebrates ^c	17.7	1322	2.0	141 ^d
Unionid clams ^e	21.4	1598	2.9	207
White suckers gill ^e	21.4	1598	2.9	207
White suckers, carcass	3.1	231	1.1	78
Days 14–24				
Water (ng·L ⁻¹)	1.0 (1.4–0.6)		2.3 (3.1–1.6)	
Biota (ng·g ⁻¹)				
Predicted	0.75		0.02	
Invertebrates	1.6	1640	0.6	261
Unionid clams	0.9	900	0.5	217
White suckers, gill	2.1	2100	1.3	565
White suckers, carcass	1.0	500	0.4	174
Days 0–104				
Water (ng·L ⁻¹)	0.1		0.3	
Biota (ng·g ⁻¹)				
Predicted	0.07		0.0002	
White suckers, gill	3.5	35 000	2.8	9333
White suckers, carcass	1.8	18 000	2.7	9000

^aTime-weighted mean concentration (total ¹⁴C corrected for extraction efficiency).

^bBased on the BCF (highest measured in Servos et al. 1989) $\times$ mean concentration in the water $\times$ fraction in true solution, i.e. 0.15 for T₄CDD and 0.01 for O₈CDD.

^cMean concentration of total ¹⁴C in benthic invertebrates.

^dBased on the mean concentration in biota divided by time-weighted mean concentration in the water.

^eAnimals exposed to sediment.

was somewhat less, the predicted biota concentration may have been overestimated. Based on the relationship between K_{oc} and K_{ow} developed by Karickhoff (1981),

$$(2) \quad K_{oc} = [\text{sediment}]/([\text{water}] f) = 0.41 K_{ow}$$

where f is the fraction of organic carbon, the predicted concentration of truly dissolved O_8 CDD in the enclosures would be only 0.004% of the total concentration in the water. This yields an even lower predicted biota concentration of $<0.004 \text{ ng} \cdot \text{g}^{-1}$ on day 10. Based on the relationship between K_{ow} and BCF developed by Veith et al. (1979), the BCF of O_8 CDD is predicted to be 4×10^6 . However, there is evidence that this relationship between K_{ow} and BCF cannot be extrapolated to superlipophilic compounds. Experimental results have shown that BCFs decline for compounds having $\log K_{ow}$ greater than approximately 6.5 (Muir et al. 1985a; Servos et al. 1989). Steric hindrances, solubility factors, and metabolic transformation may reduce the BCFs of O_8 CDD relative to those predicted by its K_{ow} (Opperhuizen et al. 1985; Gobas and Mackay 1987; Opperhuizen and Sijm 1990). Because the biota in the enclosures were exposed for only 10 d, they might not have reached equilibrium with the water. Hawker and Connell (1985) have shown that for superlipophilic compounds such as O_8 CDD, the time to reach equilibrium may be greater than the life span of the organism. In the enclosure experiments, the exposure durations were relatively short and the BCFs are therefore expected to reflect those observed in the laboratory and not that theoretically predicted by K_{ow} . For the comparisons of BCFs and predicted biota concentrations, the lipid contents of the laboratory and field organisms were assumed to be equal. Although this is a poor assumption, the lipid content of the field organisms is expected to vary by less than a factor of 10 and this does not change the general conclusions.

Biota (*H. azteca*, unionid clams, and white suckers) held in cages in the water column generally had higher concentrations of PCDDs than the biota held in cages on the sediments at the same time. This may have been due to increased sorption of PCDDs to organic matter at the sediment–water interface. Sediment interstitial waters from Lake 304 contain $28.8 \pm 3.6 \text{ mg}$ dissolved organic carbon (DOC) $\cdot \text{L}^{-1}$ compared with only $7.8 \pm 0.6 \text{ mg}$ DOC $\cdot \text{L}^{-1}$ in the water column (Servos et al. 1992). Even on day 3, PCDDs were not detectable ($<0.5 \text{ ng} \cdot \text{L}^{-1}$) in the sediment interstitial water of the lake enclosures. Based on equation (2) the concentration of T_4 CDD truly dissolved in the sediment interstitial water would be 2.3 times lower than in the water column. If the DOC–water partition coefficient (K_{doc}) measured for Lake 304 sediment interstitial water ($350 \text{ L} \cdot \text{g}^{-1}$; Servos and Muir 1989b) is used to estimate the concentration of T_4 CDD truly dissolved in water, then it is 1.5 times lower than the predicted concentration in the water column on day 10. The T_4 CDD concentration of caged biota held on the sediment on day 10 was 1.2–2.1 times higher than that of those caged in the water column. This difference was greatest for the amphipods which have a stronger association with the sediments than do fish and also a higher lipid content than fish.

Numerous studies have reported a reduction in the bioavailability of hydrophobic compounds in the presence of POM or DOM (McCarthy 1983; Landrum et al. 1987; McCarthy and Jimenez 1985; Servos and Muir 1989a). The amount of the hydrophobic compound bound to organic matter has been shown to be directly related to the organic matter concentration in solution (Landrum et al. 1984; McCarthy et al. 1985; Morehead et al. 1986; Servos et al. 1989). The organic matter –

contaminant complex is apparently too large or too polar to penetrate the respiratory membranes, or else the contaminant does not have significant time to diffuse out of the organic matter complex and interact with the biological membranes (Landrum et al. 1987). Even when the measured concentrations of truly dissolved PCDDs are used to predict their BCFs in the laboratory, they are considerably lower than would have been predicted by their K_{ow} by extrapolation of the relationship presented by Veith et al. (1979) between BCF and K_{ow} (Muir et al. 1985a; Servos et al. 1989). Failure to fully explain the divergence from the predicted relationship may be due at least partly to the difficulty of measuring the concentrations of extremely hydrophobic compounds such as O_8 CDD in true solution. Steric hindrances may also be important in controlling the uptake of PCDDs. Opperhuizen et al. (1985) predicted that hydrophobic molecules within minimum internal cross-sections (MIC) greater than $9.5 \text{ \AA}$ would not permeate the polar holes of the epithelial membranes. H_6 CDD, H_7 CDD, and O_8 CDD all have MICs of $9.8 \text{ \AA}$ (Servos et al. 1989). Gobas et al. (1988) predicted that the activity coefficients of solutes in membranes will differ from 1-octanol and that the activity coefficients will become larger with increasing solute size, likely as a result of the increased energy of cavity formation in the membranes. The reduced uptake of O_8 CDD in the enclosures might have been due to several factors which limited its uptake relative to that of the T_4 CDD. The relative importance of these factors in controlling the bioavailability of PCDDs has not yet been adequately defined, although sorption of PCDDs to organic matter plays an important role.

A possible alternative or additional explanation for the lower uptake of O_8 CDD is that the O_8 CDD in biota was derived from the food and not the water. As the concentrations of both PCDDs truly dissolved in the water declined, there may have been a shift in the route of uptake from direct equilibrium partitioning to a detrital food chain transfer. Removal of the PCDDs to the sediments may have effectively eliminated the water column as a source of PCDDs for biota. This shift may have occurred after day 24 for T_4 CDD, but the extremely low concentrations of truly dissolved O_8 CDD may have limited its bioavailability from the water column even immediately after the additions to the enclosures (when the total concentrations in the water were relatively high). The similarity in the concentrations of O_8 CDD on days 10, 24, and 104 may be due to the relatively constant concentration of O_8 CDD available to biota via the food vector (i.e. feeding). Both congeners were extremely persistent in the sediments which may have been the base of the food chain (Servos et al. 1992). The concentrations of both T_4 CDD and O_8 CDD in the digestive tract of white suckers were approximately 10 times higher than the concentrations in any other tissue. This indicates that fish were feeding on material that had relatively high concentrations of PCDDs. Although the food items were not enumerated, the digestive tract contents contained zooplankton, chironomids, and possibly sediment. The digestive tract or digestive tract contents might also have had higher lipid content which would have resulted in higher concentrations relative to the remainder of the fish tissue.

The apparent BCFs of free-swimming white suckers on day 104 (released into the enclosures on day 1) were 18 000 to 35 000 for T_4 CDD and 9000 to 9333 for O_8 CDD. This is much higher than the BCFs observed in caged fish on day 10 or 24 in the enclosures or in the laboratory (Muir et al. 1986; Servos et al. 1989). The higher BCFs could result from the longer time of exposure or from residual PCDDs accumulated during the

TABLE 6. Parameters used in the food chain model.

Compound and compartment	k_u^a (d^{-1})	k_d^a (d^{-1})	α^b (%)	F^c ($g \cdot g^{-1} \cdot d^{-1}$)
T₄CDD				
Filter-feeder	1200	0.06	6	0.105
Small fish	285	0.12	13	0.017
O₈CDD				
Filter-feeder	60	0.12	2	0.105
Small fish	30	0.08	3	0.017

^aUptake and depuration rate constants estimated from Muir et al. (1985a) and Servos et al. (1989).

^bAssimilation efficiency estimated from Muir and Yarechewski (1988).

^cFeeding rates taken from Thomann (1981), ignoring age class differences.

first few days of the experiments when concentrations in the water were relatively high. However, there was also a decrease in the ratio of the concentration of T₄CDD in the gills to the carcass between days 10 (>6) and 104 (<2). These results suggest a shift in the route of uptake of T₄CDD from the water to the food, while the major route of uptake of O₈CDD may have been food throughout the study.

Crossland et al. (1987) predicted a similar shift in the route of uptake of 2,5,4'-trichlorobiphenyl for fish in outdoor ponds. After the initial spike, when the concentrations in the water were high, the concentrations of the PCB in rainbow trout and grass carp (*Ctenopharyngodon idella*) were similar. After 14 d, the concentration of PCB in the carnivorous (invertebrates) rainbow trout was higher than in the herbivorous grass carp, indicating that uptake had shifted from uptake directly from the water to accumulation from the food. Using a steady-state food chain model, Thomann (1981) predicted that the body burden of PCBs in top predators was almost entirely due to transfer from contaminated prey. Thomann and Connolly (1984), using a similar model, predicted that >99% of the concentration of PCBs in the lake trout (*Salvelinus namaycush*) of Lake Michigan was due to exposure through the food chain. Connolly and Pedersen (1988) have calculated fugacity ratios of >1 for biota collected in the field, which also indicates that food chain transfer is an important route of exposure.

The four-compartment (steady-state) food chain model of Thomann and Connolly (1984) was used to predict the relative importance of food chain versus water uptake of PCDDs in an aquatic environment. At steady state the concentration of the PCDD in each trophic level (CF_i) is given by

$$(3) \quad CF_i = \frac{k_{ui} CW + \alpha F_i CP_{i-1}}{k_d}$$

where k_{ui} = uptake rate constant (litres per kilogram per day) from water, CW = water concentration of PCDD (nanograms per kilogram), α = assimilation efficiency from food, CP_{i-1} = concentration (nanograms per kilogram) in prey at one lower trophic level, and k_d = depuration rate constant. Values for many of the parameters are available or can be estimated from the literature (Table 6). The steady-state solution assumes that there is a single age class and no differences in the feeding rates, growth rates, or kinetic parameters within each trophic level. These assumptions are unrealistic but allow a simplistic comparison of the relative distribution of PCDDs in food chains (Thomann 1981). The relative concentrations of PCDDs derived from the water or food were predicted based on the concentrations of PCDDs measured in the sediment and

water in the enclosures (Servos et al. 1992). Uptake and depuration rate constants were taken from Muir et al. (1985b) for invertebrates and from Servos et al. (1989) for fish. Assimilation efficiencies were taken from Muir et al. (1986) and feeding rates were taken from Thomann and Connolly (1984).

The hypothesis that there is a shift in the route of uptake of T₄CDD in the lake enclosures is supported by the food chain transfer model. As the concentration of T₄CDD in the water declines, the model predicts a shift of source of accumulation from water to food, and this shift occurs sooner for the higher trophic levels (Fig. 1). Because the uptake rates of O₈CDD from water are low, a larger fraction of the O₈CDD is predicted to be derived from food, even in the filter-feeders on day 10.

T₄CDD isomers other than 2,3,7,8-T₄CDD, including 1,3,6,8-T₄CDD, are metabolized and the assimilation efficiency of the parent compound is low (Muir and Yarechewski 1988; Niimi and Oliver 1986). The assimilation efficiency of extractable 1,3,6,8-T₄CDD is <4% and it may be lower because not all of the extractable ¹⁴C remains in the fish as the parent compound (Muir et al. 1986). If this value is used in the model, then T₄CDD does not biomagnify. In contrast, 2,3,7,8-T₄CDD has a relatively high assimilation efficiency of 50% (based on data from Hawkes and Norris 1977; Kleeman et al. 1986) and a low depuration rate constant (Branson et al. 1985; Mehrle et al. 1988). Therefore, the 2,3,7,8-T₄CDD isomer will likely biomagnify up the food chain and become detectable in fish although the concentrations in detritus are very low. This might explain why 2,3,7,8-T₄CDD is often the only Tetra congener found in fish (Kuehl et al. 1987a; Rappe et al. 1987). Rappe et al. (1987) has reported finding non-2,3,7,8-substituted PCDDs in crabs. This may indicate that some invertebrates do not have the ability to metabolize PCDDs and they may bioaccumulate in these organisms. Unionid clams in this study did not appear to be able to metabolize the 1,3,6,8-T₄CDD congener. Almost all of the [¹⁴C]T₄CDD remained as the parent compound in clams, while only a small fraction of the [¹⁴C]T₄CDD in white suckers remained as the parent compound. This may have important implications for monitoring programs.

In contrast with 2,3,7,8-T₄CDD, the higher chlorinated congeners are not predicted to biomagnify (Muir and Yarechewski 1988). However, the concentration of PCDD congeners found in sediments (i.e. detritus) generally increases as the degree of chlorination increases (Czuczwa and Hites 1986). This may lead to detectable amounts of the higher chlorinated dioxins in biota despite their low assimilation efficiencies. The concentrations of the higher chlorinated PCDDs in the environment are so high that even a small amount of transfer up the food chain may result in detectable quantities in the top predators.

The concentrations of PCDDs found in biota are not directly correlated with the concentrations in the sediments, although the sediments appear to be the source of PCDDs in biota and therefore the key to predicting residue levels in the biota. Because PCDDs are extremely persistent in the environment, they may remain in the surficial sediments for a long time, depending on the physical characteristics of the system. This implies that, even if the sources of PCDDs to aquatic environments are reduced, residues of PCDDs may remain high in biota for a long period, until the PCDDs are buried in the sediments or otherwise transported out of the aquatic system.

Unfortunately, there is very little information in the literature on the relative concentrations of PCDDs in detritus and biota at different trophic levels from the same environments. The literature is dominated by monitoring studies which seldom

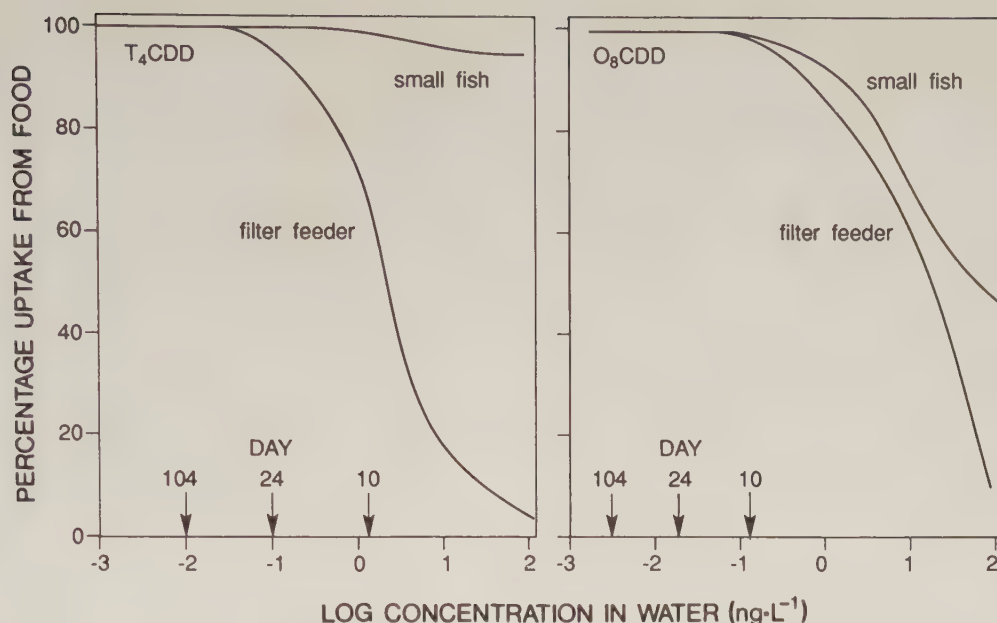


FIG. 1. Effect of PCDD concentration in water on the predicted contribution of uptake from food to the concentration of PCDDs found in biota. Predictions are based on the food chain model using parameters in Table 6 and a concentration in sediment in lake enclosures of 120 ng·g dry weight⁻¹ reported by Servos et al. (1992). Arrows represent the predicted concentrations truly dissolved in water on days 10, 24, and 104.

include both sediments and biota. Most studies on biota have concentrated on the 2,3,7,8-T₄CDD congener and rarely report the concentrations in more than one species (usually fish) from a single environment. Because of the predicted importance of food chain transfer, there is a need for a consistent data set including several trophic levels from a single environment to help clarify the distribution patterns of PCDDs observed in biota. A better understanding of the environmental dynamics of PCDDs would help to interpret the significance of residues found in biota and lead to improved assessment of the hazard they represent.

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Climatic Influence Linking Copepod Production with Strong Year-Classes in Sablefish, *Anoplopoma fimbria*

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Stomach contents from first-feeding larval sablefish (*Anoplopoma fimbria*) comprised mainly calanoid copepods. Along the west coast of Vancouver Island, these copepods were the dominant zooplankton at the depth that sablefish larvae developed. We propose that strong year-classes in sablefish populations occur when there is exceptional production of copepods. The periods of exceptional copepod production appear to be correlated with climate and ocean conditions.

On a observé que les contenus stomacaux de larves de morue charbonnière (*Anoplopoma fimbria*) en début d'alimentation comprenaient principalement des copépodes calanoides. Le long de la côte ouest de l'île de Vancouver, ces copépodes composaient la plus grande partie du zooplancton à la profondeur à laquelle ces larves de morue charbonnière se développaient. Nous pensons qu'il y a production de fortes classes d'âge dans les populations de morue charbonnière quand il y a abondance de copépodes. Le climat et les conditions océaniques semblent être responsables des périodes de production exceptionnelle de copépodes.

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Understanding the factors that control recruitment is the most important problem in fisheries science. The extensive literature published on this topic since Hjort's (1914) original hypothesis that year-class strength was determined early in the life history is evidence that the population dynamics process remains poorly understood. A major advance was made when Ricker (1954) and Beverton and Holt (1957) proposed that recruitment could be predicted from stock size. Rothschild (1986) pointed out, however, that the stock-recruitment relationship is a paradox: although the relationship of recruitment to parent stock appears to be critical to population stability, there are little supporting data for the existence of such a relationship. One difficulty with stock-recruitment theory is that the relevance of environmental factors is not well understood. By studying the relationship of environmental variation and recruitment, we attempted to gain a better comprehension of the importance of environmental variation to the stock-recruitment relationship.

In an earlier paper, we examined the relationships between the environment and recruitment of sablefish (*Anoplopoma fimbria*) (McFarlane and Beamish 1986). There, we excluded the possibility of the physical movement of larvae into more productive onshore areas because oceanographic events that were correlated with year-class strength occurred before most larval fish reached the surface waters. We hypothesized that favourable oceanographic conditions increased the amount of food available for sablefish larvae at depth. We believed that this increased production of food would coincide with the initiation of larval feeding, possibly as early as the half yolk sac utilization stage. In this study, we provide evidence to support our hypothesis.

Methods

Study Area

The study area included the La Perouse Bank area and associated slope waters off the southwest coast of Vancouver

Island (Fig. 1). Two transect lines, approximately 36 km apart, were laid out approximately perpendicular to the bottom contours. Each line extended from 8 to 200 km from shore, with sampling stations 10–20 km apart.

Sampling Procedures

Discrete depth samples were taken at each station using a 1-m² Tucker trawl that opened and closed at depth. Each trawl unit consisted of three nets. When deploying this gear, the bottom net was open with the middle and top net closed. At the desired depth, the middle net was opened and the top and bottom net closed for a tow duration of approximately 15 min. During retrieval the top net was open.

Nets were constructed of 335- μ m black nitex mesh with a ridged codend equipped with a 335- μ m screen. A flowmeter installed in the mouth of each net recorded the volume of water filtered. Samples were collected during January, February, and March 1987 and February and March 1988 at stations along the transect lines. Samples were collected at <200, 300, 500, and 700 m. In addition, Tucker trawl and surface samples were collected in April of both years from selected stations to examine the distribution and stomach contents of larval sablefish.

Surface samples were taken at night using a modified Sameoto sampler — a neuston sampler equipped with a flowmeter (Sameoto and Jaroszynski 1969). The sampler was 45 cm high $\times$ 45 cm wide with a 500- μ m mesh Nitex net. The codend was PVC with a 351- μ m stainless steel mesh window. Each tow was 15 min long at a speed of 3 knots.

The recovered nets were thoroughly washed down and the catch preserved in 5% buffered seawater formaldehyde. In the laboratory, zooplankton were identified and a subsample enumerated. Copepods from the subsample were identified to genus with the exception of calanoid copepods which were identified to species. Relative abundance estimates (numbers per cubic metre) were made for total plankton per station and for cope-

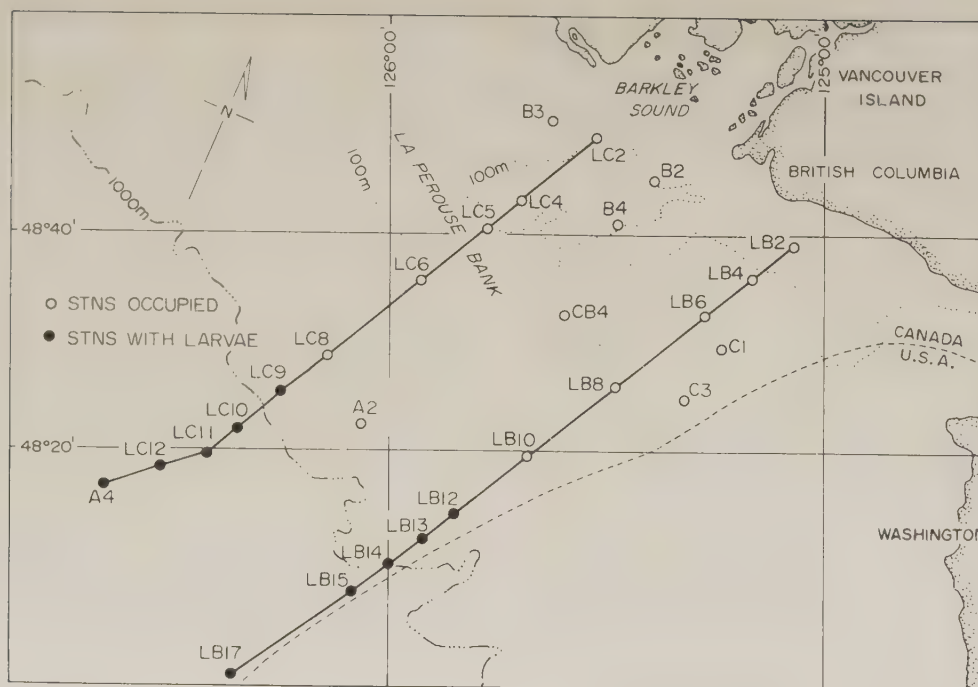


FIG. 1. La Perouse Bank survey lines and sampling stations.

Pods per station. All fish larvae were identified to species and enumerated. All sablefish larvae were measured, developmental stage determined, and stomach contents identified.

Laboratory Studies on Larval Development

In a laboratory study, sablefish were reared from egg to full yolk sac resorption (McFarlane and Nagata 1988). Information on egg and larval density relative to seawater was collected throughout the study by estimating salinity of neutral buoyancy (SNB) (Alderdice et al. 1988; G. A. McFarlane and J. O. T. Jensen, unpubl. data). Using this information, plus developmental times (McFarlane and Nagata 1988) and oceanographic data (McFarlane et al. 1991), it was possible to estimate the location of eggs and larvae by developmental stage in the water column as a function of the difference in density from that of the surrounding water (Alderdice et al. 1988; McFarlane et al. 1991). The rate of ascent or descent was calculated using Stokes' equation (Alderdice and Forrester 1971):

$$W = \frac{2}{9} g \frac{(p_1 - p_2)}{u} r^2$$

where W = vertical egg velocity (centimetres per second), g = 980.621, p_1 = egg density (grams per cubic centimetre), p_2 = density of the surrounding water (grams per cubic centimetre), u = 0.0150 (dynamic viscosity of seawater estimated at 35‰ and averaged for 5–10°C (Sverdrup et al. 1946), and r = radius of the egg (centimetres).

Schnute's (1981) general growth model was used to obtain empirical models to (1) estimate changes in egg density in relation to ambient temperature and (2) describe changes in salinity and temperature in relation to depth. This allowed calculation of water density (Millero and Poisson 1981) in the ocean water column to depths of 1000 m.

To calculate the location of eggs and larvae in the water column, these relationships were incorporated into an interactive model that, given an initial spawning depth, calculated egg density (dependent on temperature and time from fertilization).

This density value was put into the Stokes' equation to determine the vertical egg velocity at a given time and depth. The model then calculated, first at 12 h followed by 24-h intervals, the egg's new location in the water column. At this new location the water temperature and salinity were recalculated, based on the modelled STD data.

Then, the mean temperature exposure was determined and the corresponding egg development rate was calculated so that the SNB of the egg could be determined. The new SNB was again input into the Stokes' equation to calculate a new vertical egg velocity. This process was repeated until 510 h (21.5 d) past hatching.

Data Sources

The method for ageing sablefish was first developed in 1977 (Beamish and Chilton 1982). The method has since been validated (Beamish et al. 1983) and it is now known that the interpretation of annuli can be difficult for older fish. This reduced level of precision makes it difficult to identify the exact year in which previous strong year-classes were produced.

If it is assumed that total mortality has been relatively constant since the inception of the fishery and that the fishery did not target on any particular year-class, then it is possible to reconstruct relative cohort strength and develop a year-class index. Initially (McFarlane and Beamish 1983a), we reconstructed cohort strength for age frequencies of females from age composition data in 1980 and 1981 corrected for natural mortality (m) of 0.1 to identify strong year-classes in the 1950's and 1960's. This was done for the 1960–82 year-classes by standardizing the sample size for each year to 1000 individuals. The numbers of individuals in each age group were adjusted to estimate year-class strength using a natural mortality rate of 0.1. Estimates of year-class strength for year-classes from 1960 to 1975 were determined using age composition data from samples collected from 1980 to 1983. Estimates of year-class strength for year-classes from 1976 to 1982 were determined using age composition data from samples collected from 1984

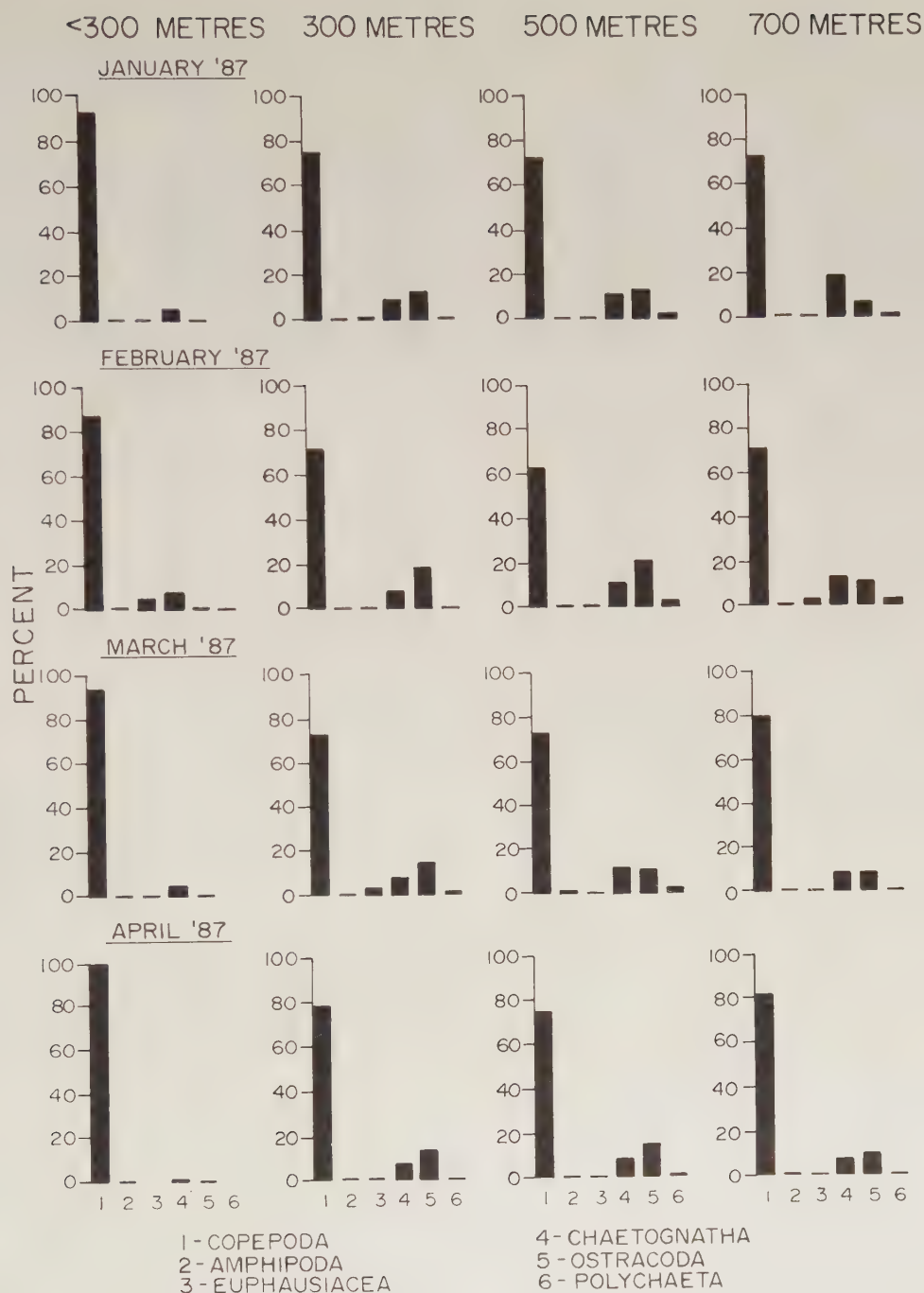


FIG. 2. Major components of zooplankton by depth, month, and year. (Fig. 2 concluded next page)

to 1989. The estimates of year-class strength were then averaged to produce a relative year-class index. The relative year-class index of year-classes in the early 1960's (1960-65) is poorly estimated because of the small numbers of observations.

Ocean Station P (50°N, 145°W) has yielded the longest time series of plankton abundance data in the central Northeast Pacific Ocean. Data for the winter months are available from 1965 to 1980, when the program was terminated. We developed an index of copepod abundance using Ocean Station P data for March-May 1965-80 (data from Fulton 1983). We chose March-May because this is the period of larval development of sablefish. A standard net haul was from 150 m to the surface at 1 m/s. Mean numbers for each month reflect the average of all vertical hauls made during daylight. In months

for which no data were available the mean number of copepods was estimated from the mean proportion of copepods captured in each month for the years 1965-80.

We needed to relate sablefish year-class strength and our copepod abundance index to an index of climate and/or ocean conditions. Because the strong 1977 year-class of sablefish occurred at the same time as strong year-classes for many other species along the west coast of North America (R. J. Beamish, unpubl. data), we believed that the environment was affecting fish production over an extremely large area. This indicated to us that the intensity of the Aleutian low pressure region, the major winter climate system in the central North Pacific Ocean, could be used as an index of climatic and oceanographic conditions. In particular, it would represent an index of wind

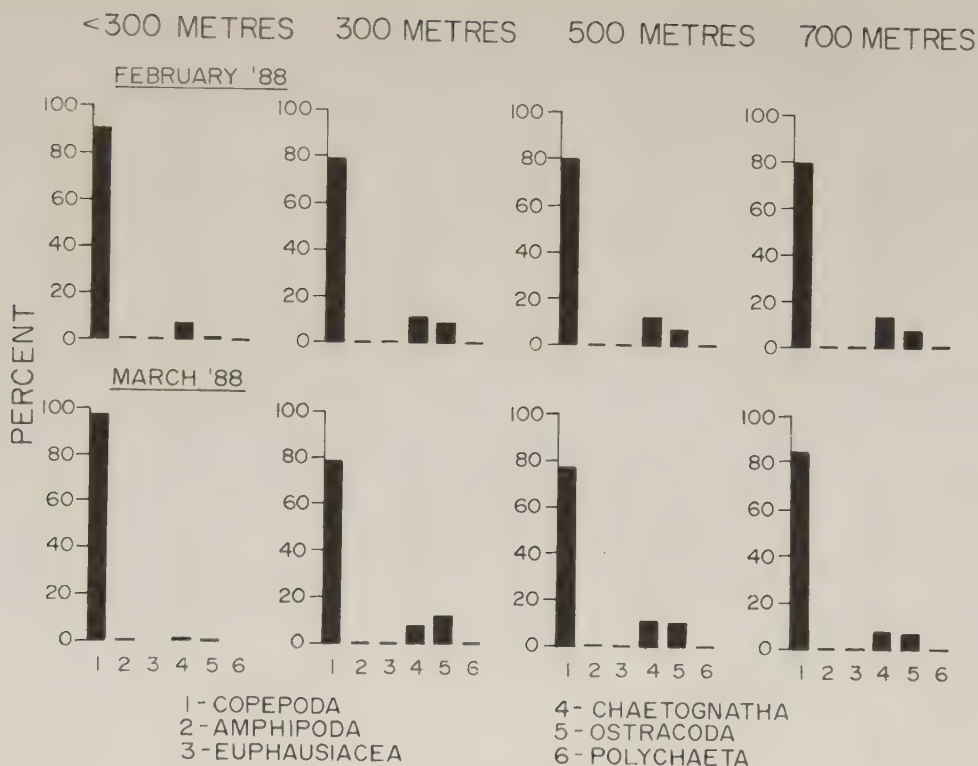


FIG. 2. (Concluded)

strength, current strength, midocean upwelling, coastal downwelling, and coastal sea surface temperature in the North Pacific Ocean.

Hamilton (1984) produced monthly estimates of the intensity of Aleutian lows in the North Pacific Ocean. We chose the 100-kPa isobar as the boundary of an intense Aleutian low. We developed an Aleutian low pressure index for winter months (December–February) for the years 1940–82 by estimating the area (square nautical miles) of the North Pacific Ocean with atmospheric pressures ≤ 100 kPa as indicated on atmospheric pressure maps (Hamilton 1984).

Results

Plankton

A total of 148 (444 samples) and 101 (303 samples) hauls were made in 1987 and 1988, respectively. In both years, copepods were the dominant zooplankton present at all depths and in all months (Fig. 2). In the upper layer (<300 m), copepods accounted for 88–98% of the plankton over all months sampled. At other depths, they accounted for 63–84%. The remaining zooplankton included representatives of Amphipoda, Euphausiacea, Chaetognatha, Ostracoda, and Polychaeta.

Copepod densities ranged from approximately 2.0 to 216.0 individuals/m³. There was large variation in the numbers of copepods per cubic metre among stations and depths (Fig. 3), with higher abundance in shallower waters (<200 m) over the continental shelf. At the stations and depths (Fig. 1) that sablefish larvae were captured (in waters >300 m over the shelf/slope break), copepods accounted for 65–92% of the plankton (Fig. 2). Calanoid copepods accounted for 85–100% of the copepods.

Larval sablefish were only captured in water deeper than 300 m in February and March of both years (Table 1). A total

of 147 sablefish larvae were captured at depth in February and March 1987 and 57 larvae in 1988. All larvae captured in February of both years were at an early developmental stage (6–8 mm; half yolk sac utilization stage or earlier). During March of both years, most (76 and 67%, respectively) larvae were captured at depths of 500 m or greater. These larvae were smaller, less pigmented, and at an earlier yolk utilization stage than those in shallower waters (<500 m). In April, no larvae were captured at depth and all larvae examined from the surface waters were >10 mm and had resorbed all yolk.

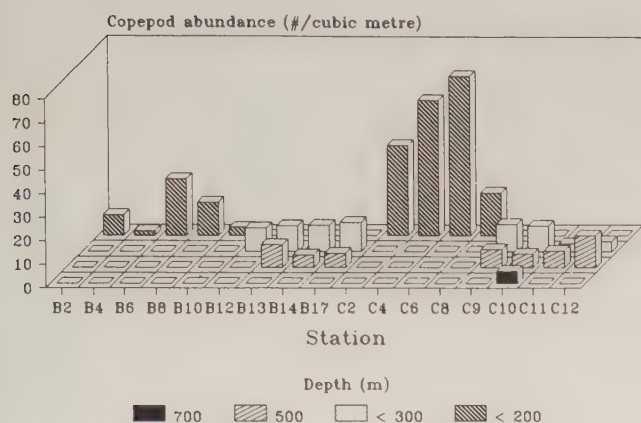
Larval Feeding

None of the larvae examined in February (Table 1) of either year contained food because they were in the early yolk utilization stage. At this stage the mouth is not functional (McFarlane and Nagata 1988). Of the 137 larvae examined from March 1987 collections, 70 (52%) contained food items. This percentage was almost identical for larvae examined from March 1988 collections (54%). In both years, sablefish larvae fed exclusively on copepod eggs and nauplii. In April, in each year, approximately 40 larvae captured in the surface waters (neuston samples) were examined for stomach contents: 85% in 1987 and 93% in 1988 contained food items. The majority (90–95%) were feeding almost exclusively on calanoid copepods (nauplii and adults).

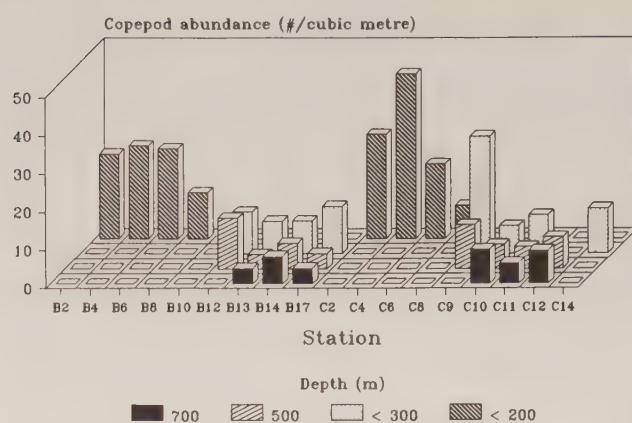
Larval Development from Laboratory Studies

Sablefish were reared from egg to full yolk sac resorption (McFarlane and Nagata 1988). Although it was difficult to make accurate SNB measurements on late-stage yolk sac larvae, visual observations of these larvae in the incubators indicate that after half yolk sac utilization, they were able to maintain themselves in the water column and would routinely move towards the surface. Incorporating the information on larval develop-

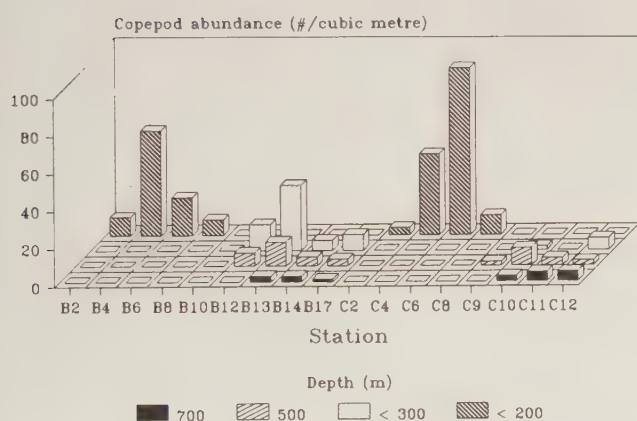
January 1987



February 1987



March 1987



April 1987

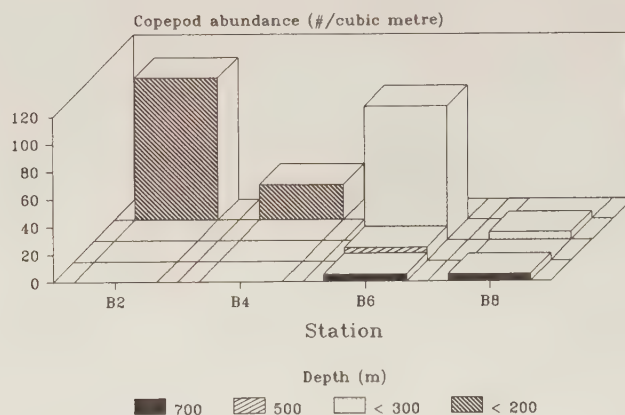


FIG. 3. Copepod abundance by month, station, and depth from 1987 and 1988. (Fig. 3 concluded next page)

ment (McFarlane and Nagata 1988), SNB, and ocean temperature at depth (McFarlane et al. 1991) into an interactive model, we can estimate development time at depth in the open ocean (Fig. 4). Assuming a spawning depth of approximately 300–500 m, the eggs rise to between 200 and 300 m immediately after fertilization (McFarlane and Nagata 1988). They remain suspended at this depth until approximately 24 h prior to hatching (12 d at 6°C). At this time, egg density increases and the eggs begin to sink. The newly hatched larvae become denser and continue sinking in the water column during early development (approximately 6 or 7 d), reaching a maximum depth of between 1000 and 1200 m. At this time, the eyes become pigmented. They remain at this depth until the half yolk sac utilization stage (approximately 14–17 d after hatching). During this time the alimentary canal, head, and body become pigmented. At approximately half yolk sac utilization, the mouth becomes functional and the larvae begin swimming movements. Larvae begin to show prey capture movements and ingest small food organisms within 2–3 d (approximately 20 d after hatching). Larvae begin to rise in the water column, presumably following copepod nauplii, and by full yolk sac utilization (approximately 40 d after hatching) are near the surface waters.

Strong Year-Classes

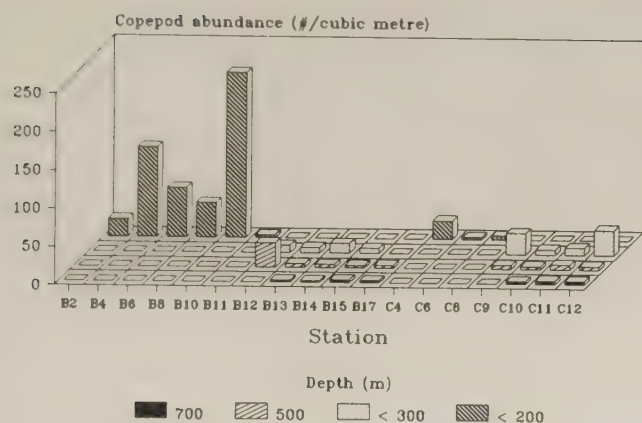
Variability in year-class survival is an important feature of sablefish populations (McFarlane and Beamish 1983a). Unfor-

tunately, age estimates needed to identify year-class strength or to reconstruct stock age compositions were not available until 1978 when the method for age determination of sablefish was first developed (Beamish and Chilton 1982). We used catch information from the commercial fishery and research cruises to identify the approximate year of strong year-classes in the 1940's, 1950's, and 1960's.

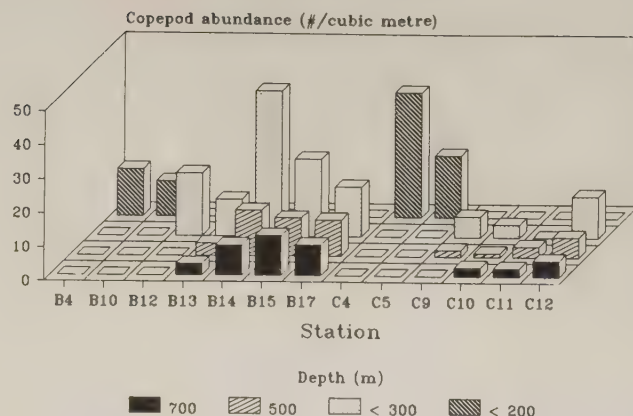
The Strong 1941 Year-Class

Exploratory groundfish research cruises started on the west coast of Vancouver Island in the mid-1940's (Forester 1946). An exploratory sunken gillnet fishery in Barkley Sound (Fig. 1) from 1944 to 1946 showed a dramatic decline in abundance of sablefish in July and August 1945 and 1946 from catches in 1944. Although no length data were available from catches in the 1940's, we believe that the method, area, and depth of capture indicate that the large numbers of sablefish caught in 1944 were juveniles. The decline in catch-per-unit-effort (CPUE) from 82.9 to 1.0 fish·net⁻¹·24 h⁻¹ in 1945 and 0.4 fish·net⁻¹·24 h⁻¹ in 1946 indicates a movement of juveniles out of this area. A similar movement of juveniles of the 1977 year-class out of shallow water occurred in the Canadian zone in 1980 (McFarlane and Beamish 1983b). If the juveniles in Barkley Sound in 1944 behaved in a similar manner, they would be age 3+ when movement occurred which suggests the 1941

February 1988



March 1988



April 1988

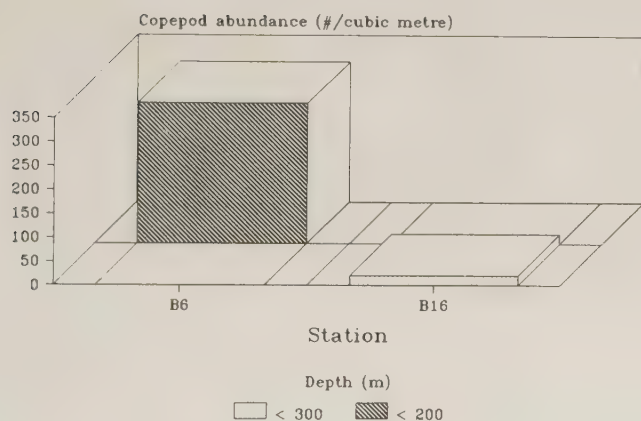


FIG. 3. (Concluded)

year-class was exceptionally strong. Another indication that large numbers of juveniles occurred in shallow waters in the mid-1940's was the introduction of a size limit in 1945 to restrict the number of small sablefish that were being caught in shallow water (Ketchen 1956). A decline in CPUE also occurred in the fisheries off Alaska between 1945 and 1947, suggesting to us that juveniles were very abundant in shallow waters prior to this time and that a strong 1941 year-class occurred over a relatively large area.

The 1953, 1958, 1967, and 1977 Year-Classes

Because sablefish live up to 70 yr (Beamish and Chilton 1982; Beamish and McFarlane 1987) the age composition of a population provides an index of relative year-class strength. We interpreted the relative year-class strength to indicate that strong year-classes occurred in the early 1950's (1953), the late 1950's (1958), and possibly the late 1960's (1967). Our initial reconstruction of relative year-class strength (McFarlane and Beamish 1983a) indicated that the 1958 year-class was exceptionally strong. We believe that this year-class was an important component of the large foreign fishery that occurred off Canada during 1968–76 (McFarlane and Beamish 1983c).

Small sablefish were abundant in the trawl fishery catches in 1969 and 1970. The large catches of small fish resulted in an attempt to develop a market for these fish during 1970

(McFarlane and Beamish 1983c). Again, assuming that these fish were approximately 3 yr old, they would be from the 1967 year-class. The most recent strong year-class occurred in 1977 (Fig. 5; Table 2). The initial indication of the strength of this year-class was the high abundance of juveniles in the inside waters throughout the range of sablefish in 1978 and 1979 (McFarlane and Beamish 1983b; Umeda et al. 1983; Fujioka 1985; Sasaki 1985). After this year-class moved out of the inside waters in 1980–81 (McFarlane and Beamish 1983b), there has been no indication of another strong year-class. The 1977 year-class was dominant in the fishery through the early to mid-1980's (Saunders et al. 1987). Although no year-classes of comparable size to 1977 have been identified since, survival of some year-classes has been above average (Francis 1985; Fujioka 1985, Saunders et al. 1987). Our relative year-class index also indicates that year-classes after 1977 were reasonably strong.

Climate, Oceanography, and Copepod Production

Strong year-classes occurred after extended periods of below-average sea surface temperatures in coastal waters which occurred off Vancouver Island during 1936–39, 1945–52, 1954–57, and 1968–76 and a moderate cooling during 1964–66 (Fig. 6). All strong year-classes followed immediately after the cooling period in coastal waters changed to a period of

TABLE 1. Sablefish larvae captured by Tucker trawl, February and March 1987 and 1988. Numbers in parentheses indicate larvae with copepods in gut.

Stn. No.	Depth strata (m)			Depth strata (m)		
	300	500	700	300	500	700
February 1987						
LB12	1	4		4	4	
LB13			1		13	10
LB14		1		3	3	1
LB17	1		1	1	5	5
LC9		1		6	4	
LC10				8	13	14
LC11				5	4	5
A4				6	5	9
Total	2 (0) ^a	6 (0) ^a	2 (0) ^a	33 (24) ^b	51 (27) ^c	53 (19) ^d
February 1988						
LB12				3		
LB13				5	3	4
LB14	1			1		2
LB15				4	1	1
LB17					2	4
LC9					5	
LC10	2	1		4	1	5
LC11				1	4	
LC12				1		3
A4			1			
Total	3 (0) ^a	1 (0) ^a	1 (0) ^a	17 (12) ^b	16 (7) ^c	19 (9) ^d

^aAll larvae at half yolk sac utilization stage or earlier.

^bMost larvae nearing full yolk sac utilization stage.

^cMost larvae at three quarters yolk sac utilization stage or later.

^dMost larvae at half yolk sac utilization stage or later.

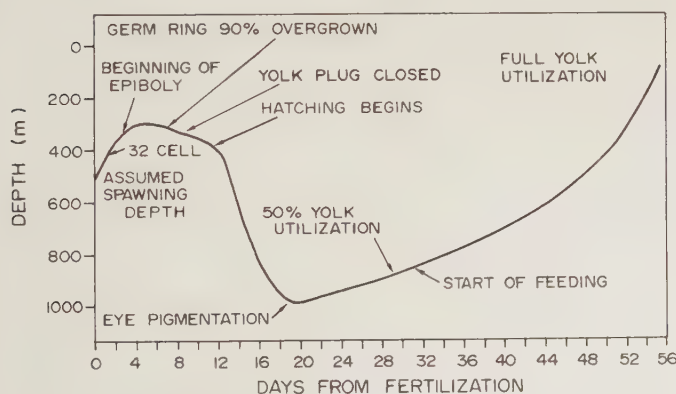


FIG. 4. Probable depth distribution by developmental stage of sablefish eggs and larvae in the study area.

above-average sea surface temperatures. There was no such period of change that did not produce a strong year-class, and no strong year-class was produced during other periods. The warming event in coastal waters following the period of change was characterized by more intense Aleutian lows in the North Pacific Ocean (Fig. 7).

The Aleutian low-pressure region is the dominant feature of the climate and ocean circulation (Fig. 8) in the northern North Pacific Ocean (Wilson and Overland 1987). Aleutian lows begin to form about the time of the fall transition in October and persist until approximately April of the following year.

Aleutian lows are caused by intense storms that frequent the area. These storms transfer momentum to surface waters increasing wave and current activity. Strong Aleutian lows intensify the strength of the eastward flowing subarctic current, the westwind drift, and the Alaska current that flows poleward (Emery and Hamilton 1985). This leads to a stronger than normal poleward advection of warm coastal water along the west coast of North America. Surface temperature patterns off British Columbia clearly show these warming trends (Fig. 6). During the winter of 1977, the Aleutian low was the strongest since 1940 (Fig. 7) and coastal sea surface temperatures were the highest since 1968. Other periods of intense Aleutian lows coincided with each of the strong year-classes identified except for the intense Aleutian lows of the early 1960's and 1970 which were not coincident with a period of below-average sea surface temperatures.

Copepods dominate the zooplankton biomass of the Northeast Pacific Ocean. Two large calanoid copepods, *Neocalanus plumchrus* and *N. cristatus*, make up 75% of the total zooplankton biomass (Miller et al. 1984). Although annual variations in copepod biomass in the Northeast Pacific Ocean are not well documented, relative abundance indices developed from Ocean Station P data (Table 3) suggest considerable variation. Despite this variation, it is apparent that biomass of copepods increased in 1976 and 1977. Copepod production was also above average in 1967.

As part of our study of sablefish year-class production, we wanted to examine the relationship among the Aleutian low index, copepod abundance, and sablefish year-class strength. Proper analysis of these data required a time series approach. Using a transfer function noise (TFN) model (Noakes et al. 1987), we found that the time series of copepod abundance (Table 3) was positively related ($r^2 = 0.63$; $p < 0.001$) to the time series of the Aleutian low index (Fig. 7). As suggested by this analysis, these two time series were sufficiently correlated that only one of them was required to adequately explain the time series of sablefish year-class relative abundance; thus, using the TFN process, we found that the Aleutian low index bore a closer statistical relationship to the sablefish year-class index ($r^2 = 0.53$; $p \leq 0.001$) than did copepod abundance ($r^2 = 0.45$; $p \leq 0.001$). These analyses demonstrated a positive relationship between sablefish year-class strength and both copepod abundance and the intensity of the Aleutian lows, with stronger year-classes tending to occur during years of more intensive Aleutian lows and higher copepod abundance.

Discussion

Theory

Strong year-classes are an important feature of sablefish population dynamics. We propose that strong year-classes are associated with large-scale increases in copepod abundance, which occur during major climate events in the North Pacific Ocean. We propose that cooling of the central North Pacific Ocean increases nutrient supply to the surface waters, improving primary productivity. The intense Aleutian low that follows this period of cooling transports nutrients and plankton into coastal waters by increasing wind-driven transport of surface waters. The intensification of the Aleutian low also increases the intensity of the northward flowing coastal current (Alaska current) and increases coastal sea surface temperature. Exceptionally strong year-classes are produced at the beginning of a cycle when coastal waters change from below-average to above-average sea surface temperatures. At the same time the

TABLE 2. Year-class index (YCI) for sablefish (1960–82) developed from the numbers in each age group of age composition samples collected from 1980 to 1989 (standardized to 1000 and adjusted using a natural mortality rate of 0.1).

Year-class	Year										YCI
	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	
1960	165	142	50	65							105
1961	156	114	88	70							107
1962	146	132	77	74							107
1963	162	117	70	82							108
1964	178	130	92	75							119
1965	134	136	70	60							100
1966	135	137	87	80							110
1967	181	167	145	98							148
1968	128	104	95	85							103
1969	97	122	107	74							100
1970	62	71	91	50							69
1971	54	63	105	64							72
1972	44	45	64	31							46
1973	38	32	77	57							51
1974	31	59	62	36							47
1975	20	51	53	34							40
1976		84	104	77							80
1977			228	272	333	214					262
1978				94	194	228	202				179
1979				144	201	186	192				181
1980					127	150	192	222			173
1981						112	112	190	183		149
1982							46	111	112	66	84

intense Aleutian low increases upwelling in the central North Pacific Ocean, further increasing transport of nutrients into surface waters. The advection of increased amounts of nutrients and plankton to and along the coast increases the survival of copepod nauplii, increasing the food supply for larval sablefish. In the following discussion, we provide evidence to support our theory.

1. Importance of spawning biology and egg and larval development

Sablefish spawn from January to March along the continental slope at depths exceeding 300 m. Peak spawning occurs in mid-to late February. Sablefish spawn almost simultaneously in all areas over the Canadian continental slope (Mason et al. 1983). Tagging studies (Beamish and McFarlane 1983, 1988; Dark 1983) indicate little movement of adult sablefish. For example, 81% of the fish tagged from February to September 1977–80 were captured in spawning condition within 100 km of the capture and release location (Mason et al. 1983), indicating no extensive spawning migration.

Eggs hatch in deep water in approximately 12 d and larvae sink to approximately 1000 m and remain there until initiation of feeding (about 20 d after hatching). Sablefish spawning is synchronous with the spawning of two of the most abundant copepods in the Northeast Pacific Ocean, *N. plumchrus* and *N. cristatus*, which constitute 75% of the zooplankton (Miller et al. 1984). We found that calanoid copepods represented 85–100% of the copepod biomass at the depths that sablefish larvae were captured. In both years the captured larvae were feeding exclusively on copepod eggs and nauplii. Therefore, it appears that larval sablefish survival is closely linked to calanoid copepod production, and a dramatic increase in copepod abundance would be expected to improve sablefish survival.

2. Importance of copepod biology

The biology and spawning behaviour of calanoid copepods are critical to our theory for the regulation of sablefish popu-

lation size. *Neocalanus plumchrus* can graze on a wide range of phytoplankton (Parsons et al. 1969) and is one of the few copepods that spawn at depths over 1000 m (Miller et al. 1984). After grazing in the surface waters during May–August, *N. plumchrus* stores lipids in preparation for overwintering. This storage of lipids during the seasonal phytoplankton production cycle not only ensures survival over the winter, but the volume of lipids is directly related to fecundity (Cooney 1987). At Ocean Station P during 1980 and 1981, Miller et al. (1984) reported that the two *Neocalanus* sp. spawned below 250 m and as deep as 1200 m during the entire year. Most spawning occurred from August through February, resulting in the production of large numbers of eggs and nauplii. After hatching, nauplii moved to the surface and copepodite stages were present in greatest abundance from April to July (Miller et al. 1984). The reproductive cycle of these two species, as determined at Ocean Station P, coincides with the spawning cycle and early development of larval sablefish. Prior to our study, there was no information on copepod reproductive behaviour or egg and nauplii development along the continental shelf or slope off the west coast of Canada. Our observations confirm the Ocean Station P observations and indicate that large numbers of copepods spawn over the Canadian continental slope at depths of at least the maximum depth sampled (700 m).

3. Relationship with climate and oceanography

We have identified a significant relationship between larval survival, copepod abundance, and environmental conditions (Aleutian lows) for the years 1965–80. During the early 1970's, a period of weak Aleutian lows and below-average sea surface temperatures in coastal waters, the year-class index clearly shows a series of weak year-classes. The relationship between Aleutian lows and copepod abundance from 1965 to 1980 suggests that larval sablefish survival improves when there is an abundant supply of copepod eggs and nauplii. The intensive

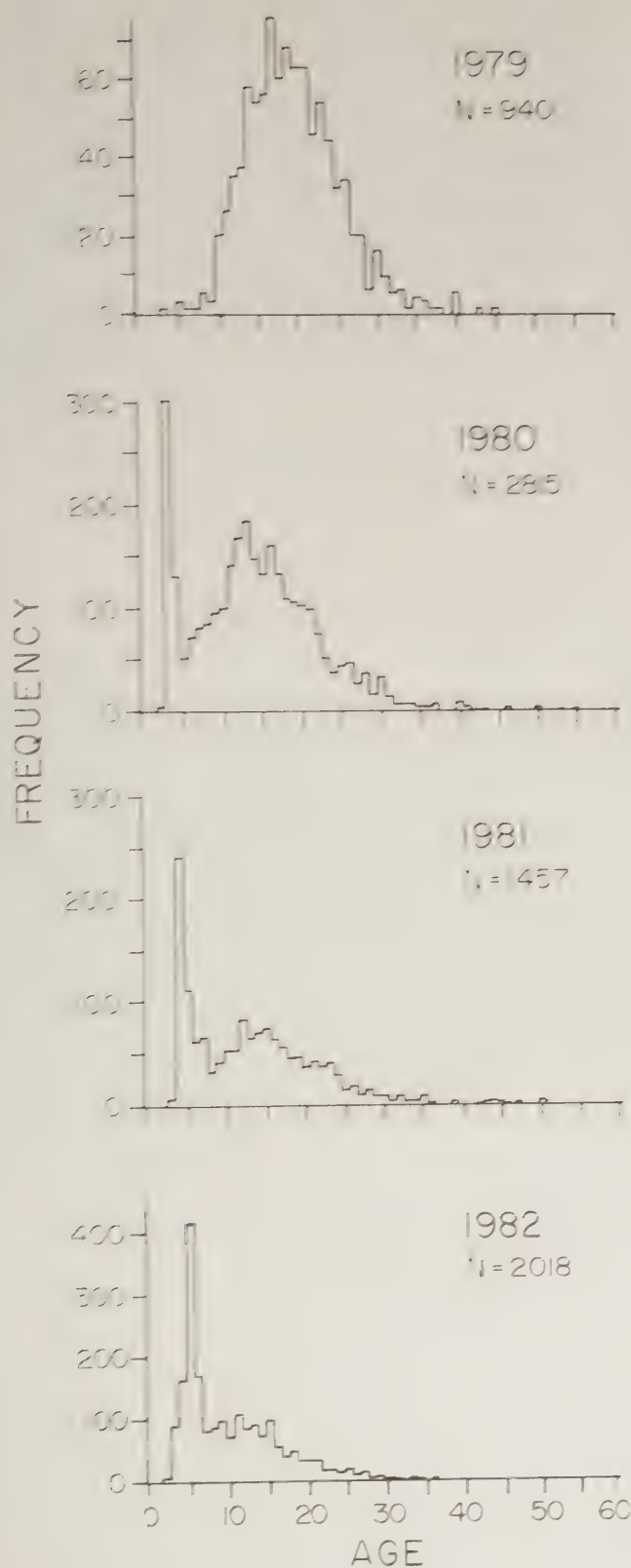


FIG. 5. Age composition of sablefish captured in the Canadian commercial fishery during 1979–82.

Aleutian lows in the early 1960's corresponded to above-average year-class strength; however, no exceptionally strong year-classes were identified. This could result because the year-class strength index was produced using age composition data from 1980 and 1981 and probably only approximates relative year-

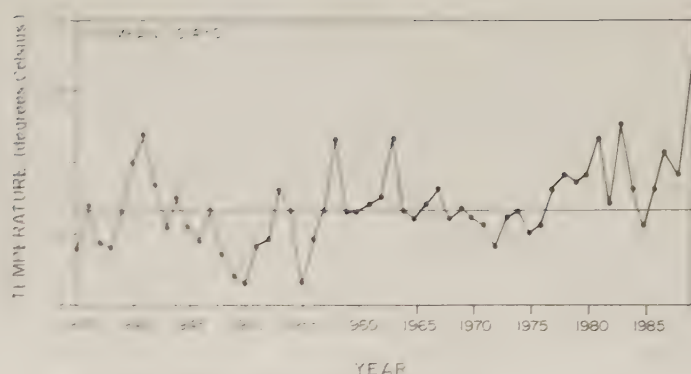


FIG. 6. Mean annual sea surface temperature at Amphitrite Point (southwest Vancouver Island) for 1935–89.

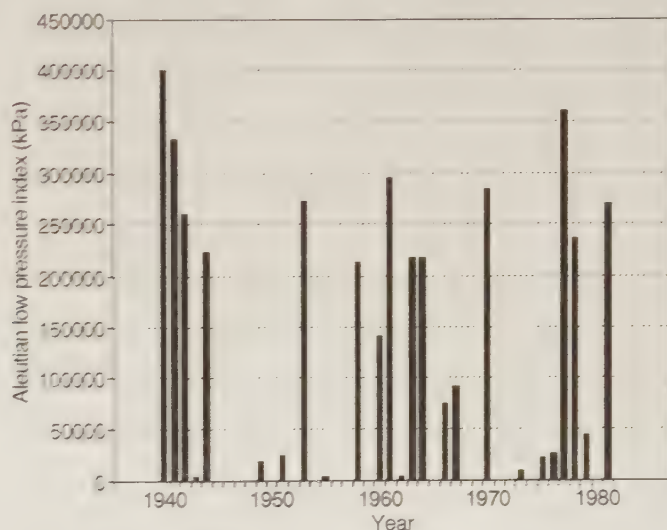


FIG. 7. Aleutian low pressure index for winter (December–February) 1940–82. The index reflects area (square nautical miles) of the North Pacific Ocean less than 100 kPa atmospheric pressure at sea surface.

class strength because of the small number of older fish in the samples. We suggest, however, that because the intense Aleutian lows in the early 1960's were not accompanied by a change from cool to warm surface temperatures in coastal waters at this time (temperatures were above average since 1958), no exceptionally strong year-classes were produced.

Climate can control ocean productivity by altering the heat storage in the surface waters above the mixing layer (Barber and Chavez 1983). A cold event is significant because during cold anomalies the thermocline is closer to the surface. This elevation brings the subsurface nutrient reservoir and surface light supply closer together, greatly increasing primary productivity (Barber and Chavez 1983). Venrick et al. (1987) identified a significant increase in primary productivity in the central North Pacific Ocean between the late 1960's/early 1970's and the early 1980's. An increase in winter winds (intense Aleutian lows) and a decrease in sea surface temperature in the central North Pacific Ocean were identified as the mechanisms responsible for this increased productivity and resulting increase in the carrying capacity. We suggest that a similar mechanism, accompanied by a change from below-average to above-average sea surface temperature in coastal waters, resulted in an increase in copepod abundance early in 1977. Conditions similar to 1977 occurred in 1940–41, 1952–53, and 1957–58 (Fig. 6, 7) and coincided with the production of the strong 1941, 1953, and 1958 year-classes of sablefish.

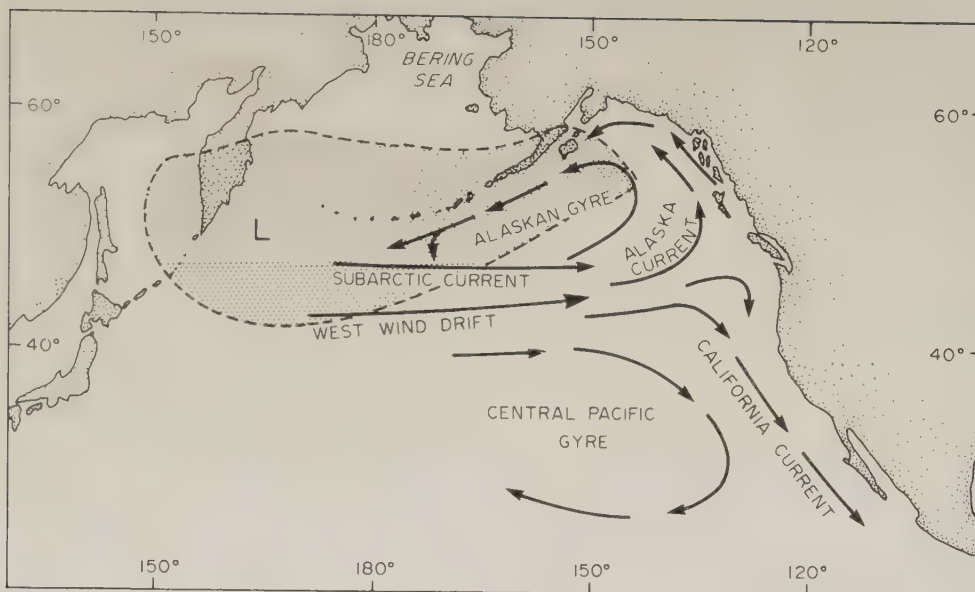


FIG. 8. Prevailing current directions and long-term mean (1940-82) Aleutian low pressure area in the North Pacific Ocean (from Hamilton 1984).

TABLE 3. Mean number of copepods per cubic metre at Ocean Station P (50°N, 145°W) in March, April, and May 1965-80.

Year	March	April	May	Average (weighted)
1965	26.5 ^a	50.8	61.4	46.2
1966	73.2	171.4	393.7	158.5
1967	81.1 ^a	98.7	246.0	141.6
1968	55.2	98.7	90.4	68.5
1969	19.7 ^a	39.8	46.8	35.4
1970	59.7	153.8	195.6	178.8
1971	4.8	14.9	25.2	15.4
1972	17.5	36.4	94.1	48.4
1973	71.2 ^a	121.3	180.2	124.2
1974	55.1	29.2 ^a	10.3	31.5
1975	44.1	84.6	105.4	92.7
1976	101.2	124.5 ^a	177.3	134.3
1977	143.2	256.2	263.4	236.3
1978	69.2	88.8	140.5	124.7
1979	44.6	41.1	231.4	106.4
1980	29.8	60.3	175.0	107.4

^aNo data available; estimated from the mean proportion of copepods caught in other months of the March-May period.

After 1977, sablefish year-classes had above-average survival (Francis 1985; Fujioka 1985; Saunders et al. 1987), but no exceptionally strong year-classes have been identified. These above-average year-classes appear to be a result of the increased productivity of the North Pacific Ocean in the late 1970's to mid-1980's identified by Venrick et al. (1987). Coastal sea surface temperatures have remained above average during this time. The absence of exceptionally strong year-classes corresponds with the absence of all ocean conditions similar to those that produced previous strong year-classes.

Many fish species exhibited exceptional production along the coast of North America from 1976 to 1978 (R. J. Beamish, unpubl. data). The increased survival of these species was also attributed to increased copepod production. Production of many of these stocks, particularly salmon, remained at a higher level in the 1980's. This increased fish production coupled with increased copepod abundance indicates that the increased sable-

fish production observed from the late 1970's to the early 1980's was part of an overall increased productivity in the North Pacific Ocean.

Frost et al. (1983) showed that calanoid copepods responded to increases in primary production by proportionally larger increases in feeding rate. An increase in feeding rate could result in increased growth, egg production, and survival in those species. Increased copepod abundance could occur as a result of improved fecundity, improved survival of copepod nauplii in coastal areas, or transport into these areas. It is apparent that the process responsible for improved copepod abundance requires further study. However, it appears that large-scale climate patterns are related to copepod abundance that in turn results in fluctuations in sablefish year-class strength.

Initially, we were concerned that our hypothesis would not explain why a relatively large population of sablefish occurred off California because *N. plumchrus* and *N. cristatus* are a minor component of the plankton in this region (Fulton and LeBrasseur 1985). Extensive surveys also have shown that larval sablefish are not found in this region (P. Smith, N.M.F.S., Southwest Fisheries Center, La Jolla, CA, pers. comm.), indicating that recruitment to this area must come from larval sablefish reared in more northern areas. Therefore, in all areas the distribution of sablefish larvae corresponds to the known distribution of *N. cristatus* and *N. plumchrus*.

Production of strong year-classes in long-lived fishes appears to be an adaptation to variations in ocean productivity or copepod production or both. Prior to the commercial fishery, sablefish probably responded to fluctuating food supplies by ensuring that there was always a minimal number of eggs produced. For sablefish and other long-lived species, this minimal number would be a function of fecundity, longevity, and population size. Over evolutionary time the length of less favourable environmental periods could select for the longevity of sablefish.

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Seasonal Reproduction and Plasma Levels of Sex Steroids and Vitellogenin in Atlantic Halibut (*Hippoglossus hippoglossus*)

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Atlantic halibut (*Hippoglossus hippoglossus*) collected off Newfoundland first mature at about 80 cm fork length (FL) for males and about 115–120 cm FL for females. Captive Newfoundland halibut did not release milt or eggs or have detectable levels of estradiol-17 β or 11-ketotestosterone until exceeding 80 cm (males) and 115–120 cm (females). Estradiol-17 β and testosterone increased to highest levels in females during gonadal recrudescence before spawning. Lower levels were observed in spawning fish. Vitellogenin (VTG) levels were highest in spawning fish. A sudden drop in estradiol-17 β and VTG preceded release of the first batch of eggs. Estradiol-17 β , testosterone, and VTG fluctuated with release of successive batches of eggs. Male halibut started to mature during fall and early winter, as indicated by increased testosterone and 11-ketotestosterone and abdominal swelling. Milt was first released in January and February when testosterone and 11-ketotestosterone were near maximum levels. Hence, rising levels of plasma sex steroids and VTG in fall indicate that reproductive activity is underway 1–2 mo before any noticeable swelling of the abdomen. Individual maturing halibut can be sexed by rising levels of estradiol-17 β and VTG (females) and 11-ketotestosterone (males) in late fall and early winter.

Le flétan de l'Atlantique (*Hippoglossus hippoglossus*) pêché au large de Terre-Neuve atteint la maturité à une longueur à la fourche (LF) d'environ 80 cm pour les mâles et d'environ 115–120 cm LF pour les femelles. Des flétans captifs à Terre-Neuve n'ont pas émis de laitance ni pondu d'oeufs, et ne présentaient pas de teneur décelable d'estradiol-17 β ni de 11-cétotestostérone avant qu'ils n'atteignent 80 cm (mâles) et 115–120 cm (femelles). La teneur en estradiol-17 β et en testostérone atteignant ses valeurs les plus élevées chez les femelles au cours du développement gonadal avant le frai. Les plus faibles teneurs étaient observées chez les géniteurs. Les teneurs en vitellogénine (VTG) étaient les plus élevées chez les géniteurs. Une baisse soudaine d'estradiol-17 β et de VTG précédait la ponte du premier lot d'oeufs. Les teneurs en estradiol-17 β , en testostérone et en VTG variaient avec la ponte des lots successifs d'oeufs. Le flétan mâle commençait à devenir mature pendant l'automne et vers le début de l'hiver, comme l'indiquait l'augmentation de la testostérone et de la 11-cétotestostérone, ainsi qu'un gonflement abdominal. La laitance était d'abord émise en janvier et en février, quand les teneurs en testostérone et en 11-cétotestostérone étaient les plus élevées. Donc, des augmentations des stéroïdes sexuels et de la VTG du plasma en automne indiquent que l'activité reproductrice est amorcée 1 à 2 mo avant qu'on puisse noter le gonflement de l'abdomen. Les flétans atteignant la maturité peuvent être triés selon le sexe par la mesure des teneurs croissantes d'estradiol-17 β et de VTG (femelles) ou de 11-cétotestostérone (mâles) vers la fin de l'automne et le début de l'hiver.

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Determination of size at first maturity, sex, and a knowledge of the time of spawning aid in the efficient management of captive fish including Atlantic halibut (*Hippoglossus hippoglossus*). Seasonal plasma steroid hormone and vitellogenin (VTG) profiles, which can be obtained by sequential blood sampling, are associated with the onset of seasonal reproductive activity and may also be used to indicate the extent of gonadal development in live fish (Crim and Glebe 1990). In females, rising levels of estradiol-17 β and testos-

terone signal active development of the ovaries, e.g. goldfish (*Carassius auratus*) (Kagawa et al. 1983). In male teleosts, growth and development of the testes is associated with rising plasma levels of testosterone and 11-ketotestosterone (Scott et al. 1980; Fostier et al. 1982).

To date, seasonal sex steroid hormone profiles have been described for only a few pleuronectids. Studies of the seasonal steroid hormone profiles of plaice (*Pleuronectes platessa*) showed that plasma levels of estradiol-17 β and testosterone

begin to rise in July, peak in February, and fall rapidly by March after spawning (Wingfield and Grimm 1977). A similar pattern was observed in male plaice where plasma testosterone levels are highest in February and decline rapidly thereafter (Wingfield and Grimm 1977). Harmin (1991) indicated that sex steroid hormones in male (testosterone and 11-ketotestosterone) and female (estradiol-17 β and testosterone) winter flounder (*Pseudopleuronectes americanus*) begin to rise during the onset of seasonal gonadal recrudescence. Hormones reach peak values in winter flounder during the prespawning period and fall rapidly to seasonal minimum values after spawning. Liu et al. (1990) reported that the presence of estradiol in plasma samples in December indicates the sex and onset of maturation of female Pacific halibut (*Hippoglossus stenolepis*). Howell and Scott (1989) demonstrated that sex steroids fluctuate daily throughout the spawning season of turbot (*Scophthalmus maximus*), a batch spawner, that spawns at 2- to 4-d intervals.

The purpose of this study was to determine seasonal changes of sex steroid hormones in relation to reproductive activities such as gonadal development and onset of spawning in captive male and female Atlantic halibut. It also compares data on size of wild-caught Newfoundland halibut at reproductive maturity with laboratory studies on captive halibut broodstock. Because captive halibut broodstock are composed of small numbers of highly valuable fish, sequential blood samples were obtained from a limited number of individuals.

Materials and Methods

Atlantic halibut were collected throughout the year by commercial vessels using longlines and by bottom trawls on Department of Fisheries and Oceans (DFO) research vessels. Halibut were collected primarily along the slope of the Grand Bank and from nearshore waters of Hermitage Bay, Port aux Basques, and Bonne Bay on the south and west coasts of Newfoundland. Most (>75%) halibut were collected at depths between 100 and 250 m and at temperatures of 3–7°C. Sex, fork length (FL, centimetres), total whole wet weight (grams), and gonad weight (grams) were determined for each halibut. A gonadosomatic index (GSI = gonad weight $\times$ 100/body weight) was calculated and plotted against fish length to determine the minimum fish length associated with the onset of gonad development. Only halibut collected during the spawning season (winter–spring, Scott and Scott 1988) were included in the plot of GSI against length (Fig. 1).

Three captive male halibut were held for several months before maturing at the Ocean Sciences Centre. Serial blood samples were collected from these laboratory-held halibut over two successive reproductive seasons. Males were monitored each month for signs of abdominal swelling, indicative of developing gonads, and to check for release of milt after apply-

ing slight pressure to the abdomen. For females, blood samples from a group of three to seven fish were collected from a well-established halibut broodstock at the Austevoll Marine Aquaculture Station, Norway. Gonadal development (size) in females was monitored by ultrasonography (Mattsson 1991). In addition, these fish were examined for external signs of abdominal swelling and, as maturation approached and the gonads grew larger, development of the ovipore. Details on sampling periodicity, halibut, and holding facilities are summarized in Table 1.

Approximately 2 mL of blood was collected with a heparinized syringe from the Duct of Cuvier (Lied et al. 1975) or from a caudal blood vessel. Blood samples were divided into aliquots and placed in Eppendorf tubes surrounded by crushed ice. Blood was centrifuged for 2 min at 12 000 g and the plasma layer removed and immediately frozen.

The sex steroid hormones estradiol-17 β , testosterone, and 11-ketotestosterone were measured by radioimmunoassay (RIA). Tritium-labelled steroids were purchased from New England Nuclear through DuPont Canada and from Amersham, UK. Anti-estradiol-17 β and anti-testosterone were obtained from ICN Immuno Biologicals, Lisle, IL. Anti-11-ketotestosterone was a gift from D. R. Idler (Ocean Sciences Centre, Memorial University of Newfoundland). Antibodies were used at final dilutions of about 1/16 of the concentration provided by ICN Immuno Biologicals for estradiol-17 β and testosterone and 1/35 000 for 11-ketotestosterone. Separation of bound and free radioactivity was achieved by addition of dextran-coated charcoal. Radioactivity was determined in a toluene-based liquid scintillation cocktail.

Steroids were extracted from thawed aliquots of plasma (50–200 μ L) by adding 1.5 mL of diethyl ether and vortex mixing for 1 min. The test tubes containing diethyl ether were placed in a dry ice – ethanol bath or in liquid nitrogen until the bottom phase was frozen. The liquid phase was decanted into a clean tube and the procedure repeated. The resulting combined ether extracts were evaporated to dryness under nitrogen in a heated water bath (20–25°C). The remaining residue was dissolved in 1 mL of absolute ethanol.

Steroid RIAs were conducted for estradiol-17 β , testosterone, and 11-ketotestosterone using the following procedures. Standard curves covering the hormone range 0–10 000 pg and halibut plasma extracts were set up in 100 μ L of absolute ethanol in duplicate tubes. After removal of the ethanol under nitrogen, 100 μ L of Tris buffer for estradiol-17 β and testosterone (0.05 M Tris-HCl, 0.1 M NaCl, 0.1% gelatin, pH 8.0) or 100 μ L of phosphate buffer for 11-ketotestosterone (0.061 M Na₂HPO₄, 0.028 M NaH₂PO₄·H₂O, 0.154 M NaCl, 0.1% gelatin, pH 7.0) was added prior to incubation of tubes at room temperature for 30 min. Next, 100 μ L of labelled steroid (approximately 10 000 cpm) and 100 μ L of antibody were

TABLE 1. Summary of sampling procedures and holding conditions for halibut.

	Males	Females
Location	Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Nfld.	Austevoll Marine Aquaculture Station, Norway
Holding facilities	Two 2-m-diameter tanks; two 5-m-diameter tanks; 3–16°C	Two 7-m-diameter tanks; 5–11°C
Sampling frequency	Approximately monthly	30–35 d; 10–15 d during spawning
Sampling duration	Sept. 1987 to June 1989	July 1988 to Apr. 1989
Number and length of halibut	3; 87–129 cm FL	3–7; 100–160 cm FL

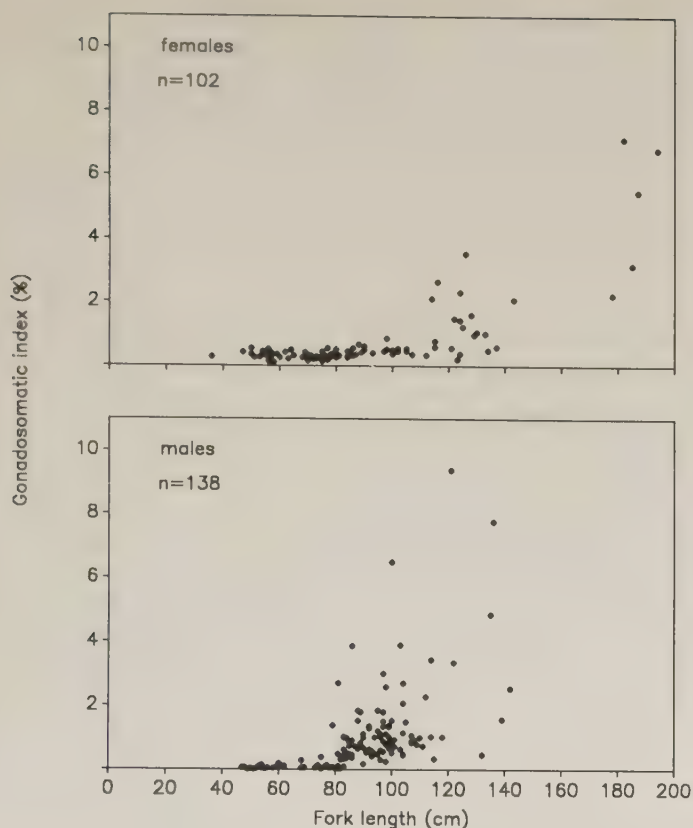


FIG. 1. GSI in relation to length of female and male halibut off Newfoundland. Only halibut collected during the winter-spring spawning season are shown.

added and the estradiol-17 β and testosterone tubes were incubated for 60 min at 37°C (11-ketotestosterone RIA incubated at 4°C for overnight). Finally, the estradiol-17 β and testosterone tubes were brought to 4°C in an ice bath and 200 μ L of dextran-charcoal (0.5% w/v Eastman Kodak Norit A activated charcoal, 0.05% w/v Dextran T-70 in Tris buffer) was added and incubated for 10 min (11-ketotestosterone RIA incubated 1 h with 600 μ L of 0.25% w/v charcoal – 0.025% w/v Dextran T-70 mixture in phosphate buffer). Charcoal pellets were precipitated by centrifugation of tubes at 3000 g and the supernatant fractions containing antibody-bound steroid radioactivity were counted for 2 min.

Interassay variation for sex steroid hormones was measured with a reference pool of halibut plasma. The interassay coefficient of variation for the plasma pool was 12.8% for estradiol-17 β ($n = 6$), 18.3% for testosterone ($n = 6$), and 16.1% for 11-ketotestosterone ($n = 3$). Recovery estimates for steroid extractions averaged 78.9% for estradiol-17 β , 85.3% for testosterone, and 77.9% for 11-ketotestosterone. Steroid specificity studies indicated that the testosterone antibody cross-reacted 1.3, 1.0, and 0.06% with estradiol-17 β , androstenedione, and 11-ketotestosterone, respectively, and <0.001% with estril, estrone, and progesterone. The estradiol-17 β antibody cross-reacted 4% with estril, 0.23% with estrone, and <0.001% with androstenedione, testosterone, 11-ketotestosterone, and progesterone. The 11-ketotestosterone antibody cross-reacted <0.1% with testosterone and 11- β -hydroxytestosterone.

Blood plasma VTG levels in female halibut were measured by homologous radioimmunoassay. VTG was purified from plasma of mature halibut by precipitation with EDTA, MgCl₂ and H₂O and anion-exchange chromatography on DEAE-Sephacel (Pharmacia Fine Chemicals) (Norberg and Haux

1985). Antisera against the purified VTG fraction were raised in rabbits. Labelling of halibut VTG was achieved by using 1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycouril (Iodo-Gen) (Sigma) as the oxidizing agent (Norberg and Haux 1988). For determination of VTG levels, a standard curve ranging from 0.05 to 10 μ g/mL was prepared in VTG buffer, i.e. 0.05 M sodium phosphate buffer, pH 7.5, containing 0.25% bovine serum albumin (Sigma). To 100 μ L duplicate aliquots of sample or standard, 20 000 cpm of label in 100 μ L of VTG buffer and 200 μ L of anti-VTG in a final dilution of 1 : 50 000 in VTG buffer were added. After 24 h of incubation at 4°C, bound and free VTG were separated by addition of anti-rabbit IgG (Sigma Immunochemicals) and the precipitate collected by centrifugation, after overnight incubation. The supernatant was discarded and the pellet solubilized in 1 mL of 5% Triton X-100 (Sigma) for 1 h. The samples were then counted in a Packard 1500 β -scintillation counter with 9 mL of scintillation cocktail (Biofluor, NEN).

Results

Halibut captured during the reproductive season off the coast of Newfoundland showed no increase in GSI until reaching a minimum length of 80 and 115–120 cm FL for males and females, respectively (Fig. 1). Field observations were supported by data from captive Newfoundland halibut which did not mature or have detectable sex steroid levels until reaching lengths greater than 80 cm (males) and 120 cm (females).

Plasma levels of estradiol-17 β in captive female halibut steadily increased from less than 3 ng/mL in July and August to greater than 20 ng/mL in mid-January (Fig. 2). After mid-January, estradiol-17 β levels declined rapidly but peaked again in mid-February. Estradiol-17 β levels declined again after the second peak to less than 4 ng/mL in late March (Fig. 2). First noticeable swelling of the ovaries occurred in early December in maturing females. The ovipore remained small and closed until a few days before the first release of eggs.

Plasma levels of testosterone in captive females followed a similar pattern to that of estradiol-17 β with the following exceptions: (i) testosterone was not detectable in July and August and levels remained low until early November, (ii) peak levels of testosterone occurred at the same time as estradiol-17 β but were lower, and (iii) levels of testosterone were the same as, or greater than, estradiol-17 β towards the end of the reproductive season in late February and March (Fig. 2).

Plasma levels of VTG in captive halibut increased slowly from July to December. Between December and January, VTG levels increased rapidly to 36.5 ± 3.3 mg/mL but rose again to levels of 47.2 ± 2.1 and 46.3 ± 16.3 mg/mL in February. Plasma VTG then declined in early March and rose to a new peak of 56.0 ± 3.7 mg/mL in mid-March (Fig. 2). Thereafter, plasma VTG levels decreased sharply to 1.6 mg/mL in April. Thus, plasma VTG levels fluctuated and reached multiple peaks during the spawning season before declining to basal values by the end of the reproductive periods.

Plasma levels of estradiol-17 β , testosterone, and VTG varied considerably from month to month in two individual female halibut (Fig. 3). Halibut 4862 was monitored daily for release of eggs throughout the spawning season. First batches of eggs were collected in early March just after estradiol-17 β , and testosterone levels dropped from greater than 30 ng/mL to less than 5 ng/mL. Sampling at 10- to 15-d intervals during the spawning season (approximately January–April) indicated the presence of multiple peaks of estradiol-17 β and testosterone as

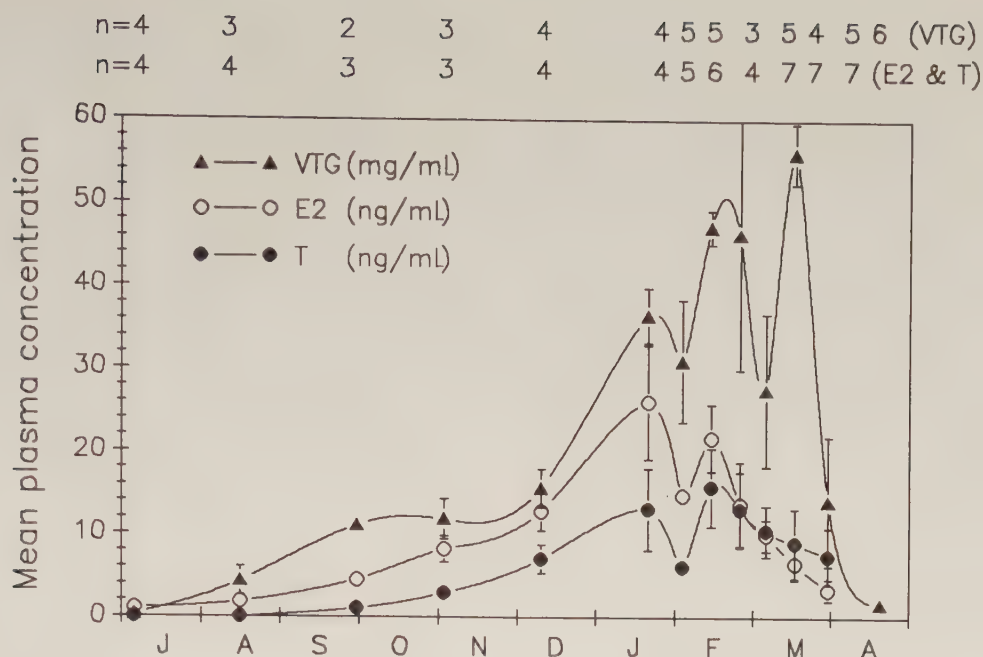


FIG. 2. Plasma levels of VTG, estradiol-17 β (E2), and testosterone (T) in laboratory-held mature female halibut prior to and during the reproductive period. Values are means $\pm$ 1 SE. Missing standard error bars are too small for presentation. *n* is the number of fish sampled.

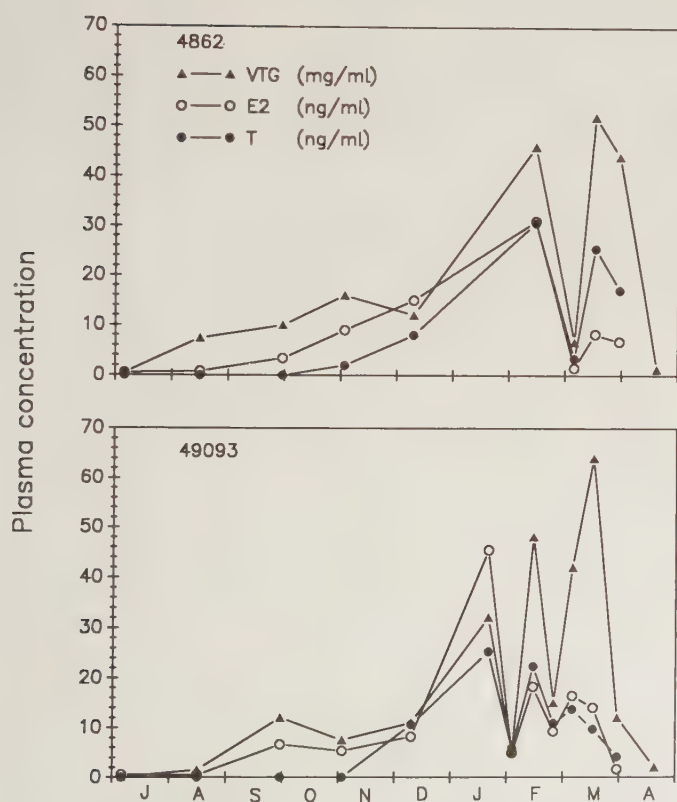


FIG. 3. Seasonal changes in plasma levels of VTG, estradiol-17 β (E2), and testosterone (T) in two captive mature female halibut (4862 and 49093).

well as VTG in these females, two peaks being present in halibut 4862 and three peaks in halibut 49093 (Fig. 3).

Testosterone and 11-ketotestosterone in captive male halibut were not detected in June, July, and August. These steroids were usually low in September, October, and November and

were highest from December to March (Fig. 4). Steroid hormone levels were low again by April.

Testosterone was detected about 1–2 mo prior to swelling of the abdomen in males. First noticeable swelling occurred from late October to early January. Release of milt first occurred in January and February, usually when steroid levels were near maximum (Fig. 4). Milt remained expressible until May or June although a progressive thickening and a diminished volume of milt was observed towards the end of the spawning season.

Discussion

Analysis of GSI and FL data indicated that 80 cm (male) and 115–120 cm (female) are the minimum lengths for sexual maturity of halibut from Newfoundland waters. These lengths are much higher than those reported by Bowering (1986) (males, 40–49 cm; females, 70–79 cm) but are similar to lengths of 50% maturity for male (87.5 cm) and female (119.5 cm) halibut caught off Newfoundland (Bowering 1986). Our observations are further supported by the lack of detectable sex steroids in captive laboratory halibut, e.g. no 11-ketotestosterone in males less than 80 cm or estradiol-17 β in females less than 120 cm in length. In a review of the biology of Atlantic halibut, Haug (1990) reported that there is considerable variation in age at first maturity and that maturity appears to be more a function of growth rate and size than age. Sexual maturity occurred at smaller sizes for Northeast Atlantic halibut collected off the Faroes Islands between 1983 and 1986: 55 cm for males and 110–115 cm for females (Jakupsstovu and Haug 1988).

RIAs for the sex steroid hormones estradiol-17 β , testosterone, and 11-ketotestosterone in plasma from laboratory-held adult halibut indicate that the seasonal hormone profile is composed of steroid levels which gradually rise in October and November, peak at different times (depending upon the individual) from December through March, and fall during and after the reproductive season to low levels in April and May.

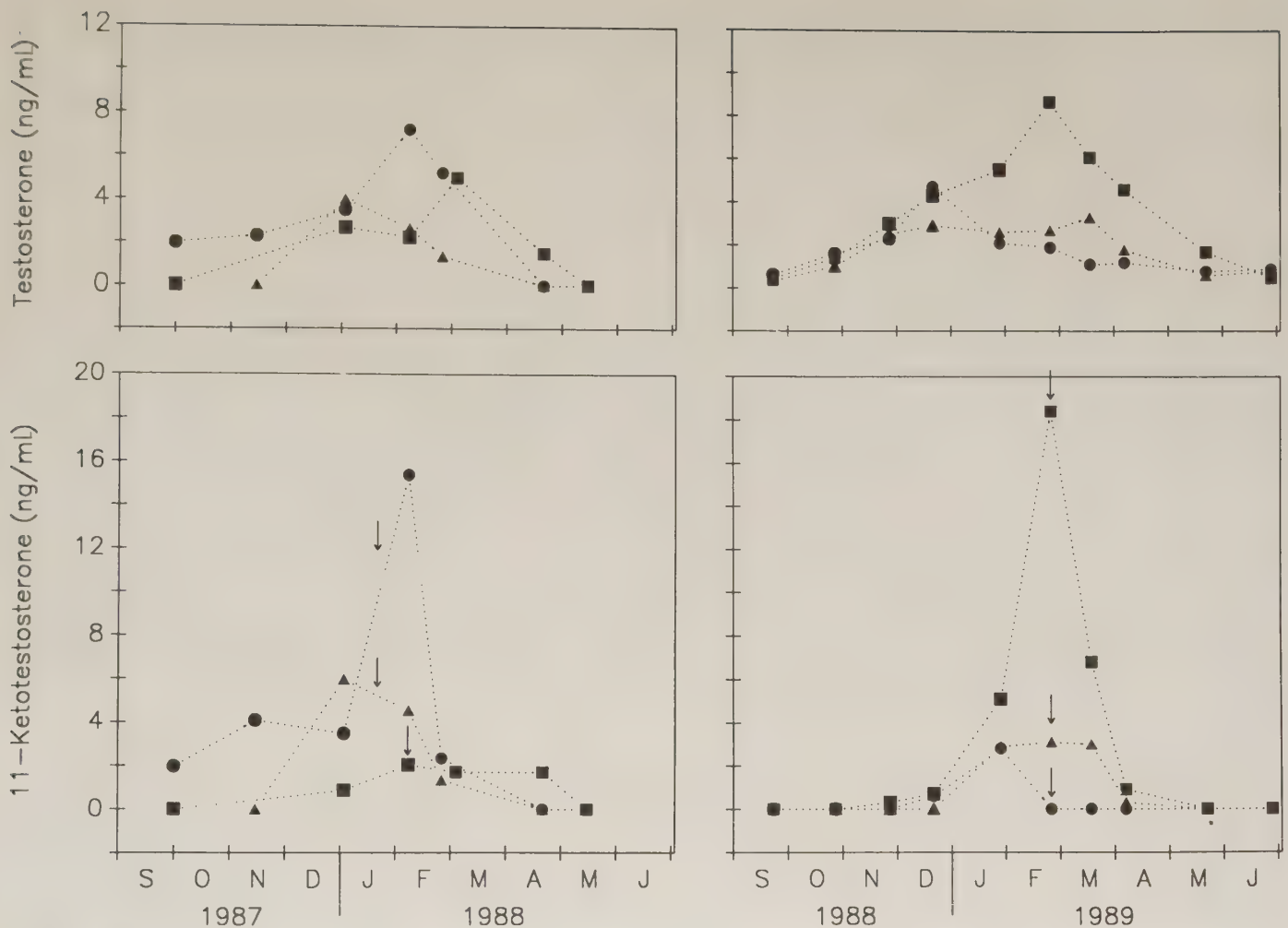


FIG. 4. Plasma levels of testosterone and 11-ketotestosterone in three laboratory-held mature male halibut over two consecutive reproductive seasons. Arrows indicate times when milt was first released. Each symbol represents a different fish.

The levels of estradiol-17 β , testosterone, and VTG fluctuate in females during the spawning season along with ovulation of successive batches of eggs. Halibut are batch spawners and can ovulate several batches of eggs within a single reproductive season (Norberg et al. 1992). We suggest that the fluctuating levels of estradiol-17 β , testosterone, and VTG may relate to cycles of oocyte clutch development as shown for goldfish, brown bullhead (*Ictalurus nebulosus*), and the killifish (*Fundulus grandis*) (Kagawa et al. 1983; Burke et al. 1984; Kobayashi et al. 1988; Greeley et al. 1988). The peak values of VTG gradually rise throughout the spawning season. One clutch of oocytes may still be vitellogenic, while another fraction undergoes final maturation. The increasingly higher levels of plasma VTG may then result from a decreased number of vitellogenic oocytes, and hence the reduced ability of the ovaries to sequester VTG, during the progress of the reproductive season, as consecutive egg batches are spawned.

A sudden drop of estradiol-17 β , testosterone, and VTG in mature female halibut appears to precede the release of the first batch of eggs. Halibut 4862 released the first batch of eggs in early March when estradiol-17 β , testosterone, and VTG decreased rapidly. This halibut (4862 = halibut 9 of Norberg et al. 1992) continued to release an additional 11 batches of eggs at a mean interval of 79 h throughout March and early April (Norberg et al. 1992). These observations suggest that halibut 49093 (Fig. 3) first ovulated eggs between 17 January

and 1 February when estradiol-17 β and testosterone levels decreased from greater than 25 to approximately 5 ng/mL and when VTG decreased from 32 to 5 mg/mL (Fig. 3).

The rising level of estradiol-17 β associated with gonadal development in the fall and the subsequent decline of estradiol-17 β in winter associated with the ovulation of the first batch of eggs may contribute to the management of halibut broodstock. Rising levels of estradiol-17 β from September to December, as distinct from the rise of plasma 11-ketotestosterone in males, indicate that ovarian development is underway in female halibut. With careful blood sampling, one can also find an initial precipitous decline of estradiol-17 β preceding the onset of spawning and the release of eggs at approximately 2- to 4-d intervals (Norberg et al. 1992). This is important because the best-quality eggs must be collected soon (hours) after ovulation (Norberg et al. 1992).

There was no evidence of multimodal androgenic steroid peaks in the seasonal profiles of testosterone and 11-ketotestosterone in male halibut. Instead, steroid levels quickly decreased from maximum levels in January and February when milt was first released. Milt release continued for several months (approximately 3–5) even after steroid levels had diminished well before the end of the spawning season. This contrasts with individual female halibut which had relatively high and fluctuating steroid levels during approximately 1 mo (Norberg et al. 1992) that eggs were released.

Future studies designed to fully characterize sex steroid hormone and VTG profiles associated with reproduction in Atlantic halibut must carefully consider increasing the frequency of blood sampling, particularly in view of the possibility that many more steroid and VTG peaks may be anticipated given that female halibut are known to spawn numerous batches of eggs within a single reproductive season.

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Evolution of Lake Whitefish (*Coregonus clupeaformis*) in North America during the Pleistocene: Evidence for a Nahanni Glacial Refuge Race in the Northern Cordillera Region

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Lake whitefish (*Coregonus clupeaformis*) could have survived through (at least) the Illinoian and Wisconsinan Pleistocene glacial maxima in an area in the vicinity of the present Nahanni National Park in the Northwest Territories of Canada according to the geological evidence. This possibility was addressed by an analysis of the genetic makeup of 43 lake whitefish populations in the Northwest Territories, the Yukon Territory, British Columbia, and Alberta. Populations in the lower Liard, Tetcela, Fraser, and upper Peace River systems as well as the headwaters of the Athabasca River were distinguished from both the Bering glacial refuge race populations inhabiting the Yukon and upper Liard River basins in the Yukon Territory and the Mississippi–Missouri glacial refuge race populations inhabiting most of the Northwest Territories, Alberta, and areas further to the east by a specific combination of electrophoretic mobility alleles. This evidence supports the hypothesis of the survival and subsequent dispersal of lake whitefish from a Nahanni glacial refugium. Possible dispersal routes and the limited extent of introgression among races are discussed.

Il est possible, d'après les données géologiques, que le Grand corégone (*Coregonus clupeaformis*) ait survécu pendant les périodes de glaciation maximale de l'illinoien et du wisconsinien (pleistocène) dans une région voisine de l'actuel parc national de la Nahanni, dans les territoires du Nord-Ouest. Cette possibilité a été examinée par une analyse du fond génétique de 43 populations de Grand corégone des territoires du Nord-Ouest, du territoire du Yukon, de la Colombie-Britannique et de l'Alberta. Les populations des bassins versants de la basse Liard, de la Tetcela, du Fraser et du cours supérieur de la rivière de la Paix, de même que celles des eaux d'amont de l'Athabasca, ont pu être distinguées de celles de la race du refuge glaciaire de Béring se trouvant dans le territoire du Yukon, et de celles du refuge glaciaire Mississippi–Missouri occupant la plus grande partie des territoires du Nord-Ouest, de l'Alberta et d'autres régions plus à l'est. La différence entre les groupes avait trait à des combinaisons d'allèles présentant une mobilité électrophorétique distincte. Cette différence appuie l'hypothèse d'une survie et d'une dispersion ultérieure du Grand corégone à partir d'un refuge glaciaire situé dans la région de la Nahanni. Les auteurs traitent des voies de dispersion possibles et de l'ampleur limitée de l'introgression entre ces races.

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The lake whitefish (*Coregonus clupeaformis*) species complex exhibits considerable and continuous morphological variation over its North American range from Alaska to Nova Scotia. Only a part of this morphological variation has been related to the isolation of stocks in separate refugia during

the Wisconsinan glaciation (Franzin and Clayton 1977; Lindsey et al. 1970; McPhail and Lindsey 1970). In contrast, the distributions of allozyme alleles are characterized by some clear discontinuities indicative of extended periods of isolation. Thus, lake whitefish populations in western Canada have been divided into two genetically distinct groups (Franzin and Clayton 1977; Lindsey et al. 1970). One population group inhabits the Yukon River system and some surrounding drainages, while the other occupies the rest of western Canada. These population groups are thought to reflect the subdivision, during the Pleistocene, of the ancestral North American lake whitefish stock by ice and

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mountains into a Bering glacial refuge race (in unglaciated parts of the Yukon Territory and Alaska) and a Mississippi–Missouri glacial refuge race (south of the continental ice sheet and east of the continental divide) during the Wisconsinan and/or earlier glacial maxima, followed by recolonization of ranges similar to the present range during periods of glacial minima.

Parts of the present Nahanni National Park in the southwest corner of the Northwest Territories were ice-free during both the Wisconsinan and Illinoian glacial periods (Ford 1976). This ice-free Nahanni region lies at the southern end of an ice-free corridor which extended northwesterly through the Mackenzie Mountains to the Alaskan North Slope (Prest 1970). It is of considerable zoogeographic interest because it may have provided a northern refuge for flora and fauna that was isolated from the Bering glacial refuge (unglaciated parts of the Yukon Territory and Alaska) by the Mackenzie Mountains. Furthermore, the Nahanni region lies at the northern end of what may have been a larger ice-free corridor extending south into Alberta (Reeves 1973, 1983). Hence, species surviving in a Nahanni glacial refugium might have had unique and early dispersal opportunities, both before and during deglaciation.

Franzin and Clayton (1977) described a genetically unique population of lake whitefish from Cache Lake (now Divide Lake), a headwater lake in the South Nahanni River system. This observation coupled with the geological history of the area raises the possibility that a third group of lake whitefish, descended from fish that survived in and subsequently dispersed from a Nahanni Glacial refugium, still exists in western Canada. If a distinct race of lake whitefish does exist in the Nahanni region, it would represent the third such race in waters of the Mackenzie River system, with Bering race whitefish in the upper Liard (Franzin and Clayton 1977) and Peel River systems (Bodaly and Lindsey 1977) and Mississippi–Missouri race lake whitefish in Great Slave and surrounding lakes (Lindsey et al. 1970).

In this paper, we report the results of biochemical genetic analyses of lake whitefish collections from the Nahanni and broadly adjacent areas in western Canada. We examine the data for evidence of (1) survival of lake whitefish in a Nahanni glacial refuge and (2) patterns of their subsequent dispersal and for (3) the extent of introgression among glacial refuge races of lake whitefish.

Methods

Fish were obtained by gillnetting at 43 sites (Fig. 1) in western Canada over the period 1968–86. They were frozen soon after capture and stored frozen at -40°C for varying periods until analysis. Eighteen of these populations were sampled for the first time in this study. Data from the remaining populations were taken from Franzin and Clayton (1977), Bodaly and Lindsey (1977), and Bodaly et al. (1992). In most (17 of 25) of these later cases, new data from additional loci and in some cases new collections were included. The source(s) of data for each population are given in Appendix 1. Hemoglobin phenotypes and the protein products of four dehydrogenase enzyme systems, glycerol-3-phosphate, isocitrate, lactate, and malate dehydrogenase (14 genetic loci), were examined (Table 1). Sample sizes ranged from 0 to 250 and averaged 39. The horizontal starch gel electrophoresis method of Tsuyuki et al. (1966) was employed. A pH 8.0 Tris–citrate buffer (Clayton et al. 1973) was used for all enzymes except MDH where a pH 6.1 amine–citrate buffer (Clayton and Tretiak 1972) was used. Electrophoresis of hemoglobins was carried out following Lindsey et al. (1970).

Table 2 gives the relative mobilities of all alleles observed. The system of nomenclature for isozymes, loci, and alleles follows Shaklee et al. (1989). Genetic models for LDH and G3PDH were described by Clayton and Franzin (1970) and Clayton et al. (1973). A model assuming two loci with two alleles at the more anodal locus and three alleles at the more cathodal locus accounts for the variation observed at sIDHP loci from liver tissue (see also Casselman et al. 1981; Vuorinen 1984). An established model for salmonid cytoplasmic malate dehydrogenase (Clayton et al. 1975; Casselman et al. 1981; Vuorinen 1984) accounts for variation observed at *sMDH-A-1,2** and *sMDH-B-1,2** loci. A genetic model for rainbow trout (*Oncorhynchus mykiss*) mitochondrial MDH (Clayton et al. 1975) has been modified in consultation with R. D. Ward, CSIRO, Hobart, Tasmania (pers. comm.), to better account for the observed phenotypes in salmonid species. The model postulates invariant alleles at the duplicated *mMDH-1** and *mMDH-2** loci and two alleles at the *mMDH-3** locus. We have yet to develop a genetic model that satisfactorily accounts for variation in hemoglobin banding patterns across all of western Canada. Hence, hemoglobin phenotypes were simply scored as two groups: “fast” and “slow,” based on their relative mobility (Lindsey et al. 1970).

For the purpose of this paper, we divide the Peace and Liard River systems into upper and lower drainages to reflect the known fish dispersal barriers of the Peace and Liard canyons (Lindsey and McPhail 1986; Fig. 1).

Results

Allele frequency data are presented in Appendix 1. Graphical representation of the data per locality, combined with all available data from across North America, is presented in a companion paper (Bodaly et al. 1992).

G3PDH-1* and G3PDH-2* Loci

The *G3PDH-1*b* allele was present in virtually all populations sampled east of the Rocky Mountains, but was absent in all populations sampled from the Fraser (sites 29–32), upper Peace (25–27), Yukon (3–8), Alsek (1–2), Peel (9), and upper Liard (10–17) River systems (Appendix 1; see also Franzin and Clayton 1977). Furthermore, this allele was absent in all lake-dwelling populations sampled in the lower Liard (19–23) and neighbouring Tetcela (24) River drainage basins, but it was present in low frequency in all populations sampled directly from the lower Liard and Mackenzie (39–42) rivers. A new, rare, and electrophoretically slow allele (*G3PDH-1*h*) was found only in the Toobally Lake population (16).

The *G3PDH-2*e* allele was absent in all lake populations of the lower Liard (19–23), Tetcela (24), Fraser (29–32), and upper Peace (25–27) River systems. It was also absent from the Talbot Lake population (33), a headwater of the Athabasca River system. In contrast, this allele was present in most populations in the Alsek, Peel, and Yukon River systems where it attains its highest frequencies (see Franzin and Clayton 1977). This allele occurs sporadically at low frequency in populations (34, 38) from the plains of western Canada (see also Bodaly et al. 1992). Outside the Yukon Territory, populations sampled directly from the Liard (18) and Mackenzie (39–42) rivers showed the highest frequencies of this allele.

Hemoglobin Phenotypes

The HB “fast” phenotype was absent in all populations sampled in the Alsek (1–2) and Yukon (5, 7–8) River systems (see



FIG. 1. Study area of western Canada, showing sample sites, river systems, and fish dispersal barriers. Numbered sites are named in Appendix 1.

also Lindsey et al. 1970; Franzin and Clayton 1977). In addition, the "fast" phenotype was absent in all upper Liard populations (10–11, 13–14), with the exception of Toobally Lake (16), where the "slow" phenotype predominated. Both phenotypes were usually found in all other populations sampled both to the south and east of the Yukon Territory, including Fisherman Lake (19) on the lower Liard River.

*slDHP-3** and *slDHP-4** Loci

The *slDHP-3*c* allele is present in low frequency in many populations of the Yukon, Alsek, and upper Liard River systems (see also Bodaly et al. 1992), but absent in all other populations with the exception of those in the lower Liard (18) and Mackenzie (42) rivers. The *slDHP-4*c* allele is found in most populations sampled from the plains of western Canada (see

also Bodaly et al. 1992), but is virtually absent in all populations sampled from the Fraser, upper Peace, lower and upper Liard, Yukon, and Alsek River systems. The *SIDHP-3*a*, *SIDHP-3*d*, and *SIDHP-4*a* alleles are found in most North American lake whitefish populations (Bodaly et al. 1992), and these were, with rare exceptions (populations 21 and 33), the only alleles found in the lake populations of the lower Liard, Tetcela, Fraser, and upper Peace systems as well as in Talbot Lake (33).

*LDH-A-2** and *LDH-B-2** Loci

The *LDH-A-2* locus is polymorphic across western Canada with no apparent pattern to the distribution of alleles. The geographic distribution of alleles at the *LDH-B-2** locus was similar to that reported previously (Franzin and Clayton 1977;

TABLE 1. List of proteins studied, their enzyme numbers where appropriate, abbreviations used, number of loci, and tissues examined (M = muscle; L = liver; B = blood).

Protein	Enzyme Commission No.	Abbreviation	No. of loci	Tissue tested
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	2	M
Isocitrate dehydrogenase	1.1.1.42	sIDHP	2	L
Lactate dehydrogenase	1.1.1.27	LDH-A	2	M
Malate dehydrogenase	1.1.1.37	LDH-B	2	M
		sMDH-A	2	L
		sMDH-B	2	M
		mMDH	3	M
Hemoglobin		HB	1	B

TABLE 2. Relative mobilities of lake whitefish allozyme alleles. Previous allele nomenclature (Franzin and Clayton 1977) is given in parentheses.

Locus	Allele	Locus	Allele
<i>G3PDH-1*</i>	<i>a</i> 100 ^a (<i>b</i> ²) <i>b</i> 65 ^a (<i>b</i> ¹) <i>d</i> 175 ^a (<i>b</i> ³) <i>h</i> 40	<i>LDH-B-2*</i>	<i>a</i> 100 ^a (<i>Hα</i> ^B) <i>b</i> 80 ^a (<i>Hα</i> ^A) <i>d</i> 112 ^a
<i>G3PDH-2*</i>	<i>a</i> 100 ^a (<i>A</i> 2) <i>e</i> 80 ^a (<i>A</i> 1)	<i>sMDH-A-1,2*</i>	<i>a</i> 100 ^a <i>b</i> 30 ^a
<i>sIDHP-3*</i>	<i>a</i> 100 ^a <i>c</i> 75 ^a <i>d</i> 125 ^a	<i>sMDH-B-1,2*</i>	<i>a</i> 120 ^a <i>b</i> 100 ^a
<i>sIDHP-4*</i>	<i>a</i> 100 ^a <i>c</i> 120 ^a <i>d</i> 135 ^a	<i>mMDH-1*</i>	<i>a</i> 100 ^a
<i>LDH-A-1*</i>	<i>a</i> 100 ^a	<i>mMDH-2*</i>	<i>a</i> 100 ^a
<i>LDH-A-2*</i>	<i>a</i> 100 ^a <i>b</i> 135 ^a <i>c</i> 45 ^a	<i>mMDH-3*</i>	<i>a</i> 100 ^a <i>b</i> 50 ^a

^aEquivalent alleles to Boday et al. (1991).

Bodaly and Lindsey 1977). All populations from the Yukon, Alsek, and Peel River systems were homozygous for the *LDH-B-2*a* allele. Similarly, nearly all populations of the upper Liard (10–15) were homozygous for this allele, except for those in Toobally (16) and Crooked (17) lakes from the lower reaches of the upper Liard, which expressed both *LDH-B-2*a* and *LDH-B-2*b* alleles. In contrast with the fixation of *LDH-B-2*a* in most upper Liard lakes, the *LDH-B-2*b* allele was fixed in many lake populations in the lower Liard (19–22) River system, but the *LDH-B-2*a* allele did occur in relatively high frequencies in populations taken directly from the lower Liard and Mackenzie (18, 39–42) rivers.

*sMDH-A-1,2** and *sMDH-B-1,2** Loci

The geographical distributions of *sMDH-A-1,2** and *sMDH-B-1,2** alleles in lake whitefish populations are complex. The *sMDH-A-1,2*b* allele was found in all populations tested in the Alsek, Yukon, Peel, and upper Liard River drainages and in one population (39) sampled directly from the Mackenzie River, but was absent elsewhere. The distribution of the *sMDH-B-1,2*b* allele was almost the exact opposite. It was absent from

TABLE 3. Distribution of allozyme alleles and hemoglobin phenotypes among races of lake whitefish in North America. Loci that express the same alleles in all three races are not shown.

Allele	Lake whitefish glacial refuge race		
	Bering	Nahanni	Mississippi–Missouri
<i>G3PDH-1*b</i>	—	—	+ ^a
<i>G3PDH-2*e</i>	+	—	+
<i>sIDHP-3*c</i>	+ ^b	—	—
<i>sIDHP-4*c</i>	—	—	+ ^c
<i>LDH-A-2*b</i>	—	+ ^d	—
<i>LDH-B-2*b</i>	—	+	+
<i>sMDH-A-1,2*b</i>	+ ^b	—	—
<i>sMDH-B-1,2*b</i>	—	+	+
<i>mMDH-3*b</i>	+ ^b	—	—
HB “fast”	—	+	+

^aUnique to Mississippi–Missouri race.

^bUnique to Bering race.

^cUnique to Mississippi–Missouri rare with race exception.

^dUnique to one population in Nahanni race.

all populations in the watersheds of the Alsek, Yukon, Peel, and upper Liard rivers (excepting Toobally and Crooked Lakes (16, 17)), but was present everywhere else. Toobally Lake, Crooked Lake, and Ft. Simpson (39) were the only populations to express both alleles.

mMDH Loci

The distribution of the *mMDH-3*b* allele coincided closely with that of the *sMDH-A-1,2*b* alleles (Table 2; Appendix 1), but was also present in populations in the Mackenzie River (40, 42), lower Liard River (18), and Cox Lake (43) in the Richardson River system.

Discussion

Lake Whitefish Races in Western Canada

Previous studies distinguished a Bering race of lake whitefish, presently found in the Yukon River basin and adjacent watersheds in northern British Columbia and central Alaska, and a Mississippi–Missouri race, found in the Mackenzie basin of the Northwest Territories and areas further east and south (Lindsey et al. 1970; Franzin and Clayton 1977; Bernatchez and Dodson 1991). The present results indicate that a third distinctive lake whitefish race inhabits waters in the southwest corner of the Northwest Territories, central British Columbia, in lakes of the lower Liard, Tetcel, Fraser, and upper Peace River systems as well as Talbot Lake (33), a headwater of the Athabasca River (Table 3; Fig. 2). We hypothesize that this race represents descendants of fish which survived one or more Pleistocene glacial periods in a refuge in the vicinity of the present day Nahanni National Park on the South Nahanni River, N.W.T. We will refer to this race as the Nahanni (glacial refuge) race.

The Nahanni race is characterized by a distinctive combination of alleles which differs from those present in either of the races previously characterized (Table 3; Fig. 2). This race is characterized by the absence of the *G3PDH-2*e* allele (present in some populations in both other races), the absence of the *sIDHP-3*c*, *mMDH-3*b*, and *sMDH-A-1,2*b* alleles (present only in the Bering race), the absence of the *G3PDH-1*b* allele and, with rare exception, the *sIDHP-4*c* allele (present only in the Mississippi–Missouri race), and the

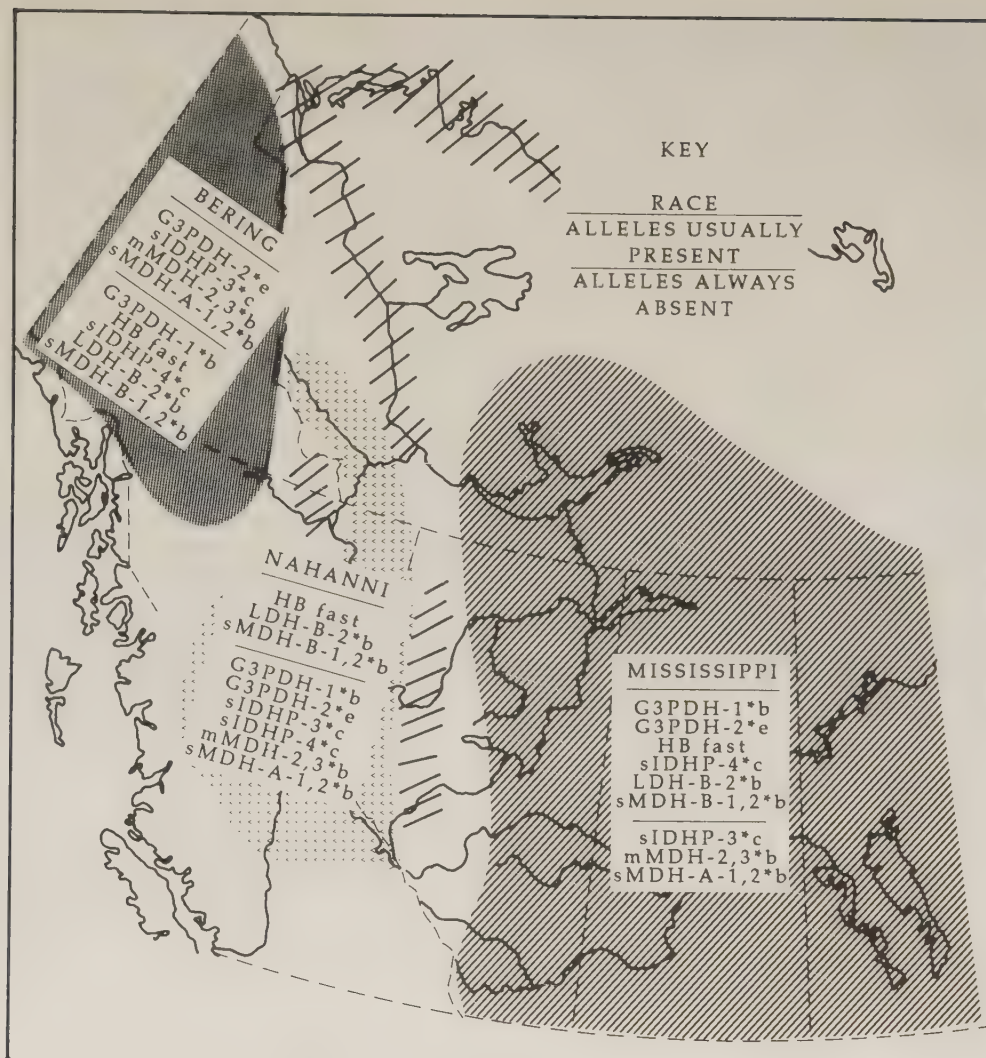


FIG. 2. Distribution and characteristic allele composition of Bering, Nahanni, and Mississippi-Missouri glacial refuge races of lake whitefish (shaded areas). Hatched areas correspond to zones of introgression among races. Blank areas correspond to areas of unknown race and, in the case of southern and western British Columbia, to areas naturally devoid of lake whitefish.

presence of the *LDH-B-2*b* and *sMDH-B-1,2*b* alleles and the "fast" HB phenotype (absent in the Bering race).

Nei's (1978) genetic distance indicates that the Nahanni race is more closely related to the Mississippi-Missouri race than it is to the Bering race (see Bodaly et al. 1992). The average genetic distance between six Bering and four Nahanni populations at all 15 loci sampled was 0.150 ± 0.007 SE. In contrast, the four Nahanni and four Mississippi-Missouri populations compared were much more similar, with an average distance of 0.047 ± 0.004 SE. Indeed, the Nahanni and Mississippi-Missouri populations were not completely separable with Nei's genetic distance alone (Foote 1980; Bodaly et al. 1992). Nei's genetic distance is not sensitive to the presence or absence of alleles per se, but rather to the absolute magnitude of allele frequency differences. Because of this sensitivity, the often large fluctuations in the frequencies of alleles common to both races (e.g. *LDH-B-2*b*, *G3PDH-1*a,c*, and *sIDHP-3*a*) tended to obscure consistent differences between the Nahanni and Mississippi-Missouri races in the presence and absence of the *G3PDH-1*b*, *SIDHP-4*c*, and *G3PDH-2*e* alleles. We believe that the distribution of shared alleles is much more important in the context of racial structure

than variation in the estimated frequencies in which given alleles occur.

The survival of lake whitefish in a Nahanni glacial refugium is supported by the existence of numerous distinct Nahanni race populations (19–23) in the very near vicinity of the glacial refuge (see Ford 1976). Indeed, Little Doctor Lake (24), which supports a typical Nahanni race population, is situated in the former outflow channel of the Nahanni glacial refuge area. Further, the unparalleled fixation of alleles at various and often different loci in the lake populations of the Nahanni region (e.g. *G3PDH-1**, *LDH-B-2**) is consistent with the presumed relatively great age of these populations and the associated effects of genetic drift. Finally, the distinctive distribution of Nahanni race lake whitefish is difficult to account for by dispersal of fish from either a Mississippi-Missouri or Bering refugium. Nahanni race lake whitefish presently occur in the watersheds of the Fraser, Skeena, and upper Peace rivers, a vast area inaccessible to most fish species dispersing from the Bering and Mississippi-Missouri refugia (McPhail and Lindsey 1970; Lindsey and McPhail 1986). Similarly, Nahanni race whitefish have also gained access to the upper Liard River watershed (16, 17), where the large majority of Mississippi-

Missouri fishes, including Mississippi–Missouri race lake whitefish, have been excluded by the rapids of the Liard Canyon (Lindsey and McPhail 1986).

There are two alternative hypotheses that could explain the present distribution of biochemical alleles: (1) for western Canada in general, the present distribution of alleles results from postglacial selection on specific alleles and (2) for the Nahanni race specifically, this group was formed by the postglacial introgression of Bering and Mississippi–Missouri race whitefish.

Franzin and Clayton (1977) argued strongly against the first alternative and their conclusions are supported here. Selection of particular alleles is usually indicated by clines in allele frequencies (e.g. Koehn 1970; Powers and Place 1978; Philipp et al. 1985; Ropson et al. 1990), and the distribution of clines usually differs among separate loci, reflecting the different selection pressures acting on different enzymes (e.g. Hunt and Selander 1973; Philipp et al. 1983, 1985; Ropson et al. 1990). However, there is no evidence of extensive gradual clines in the frequencies of any alleles in this study. Rather, there are abrupt and coincident breaks in the distribution of alleles at numerous independent loci leading to extensive geographic areas where they are either present or absent (Fig. 2; Appendix 1). Note that the breaks in allele distributions are often associated with known dispersal barriers to fish (e.g. the rapids of the Liard and Peace River canyons) suggesting that navigation barriers have played a larger role in the present distribution of alleles than selection. Supporting this, there are few significant correlations between allele frequencies and physical or environmental parameters (Franzin and Clayton 1977; Foote 1980). The only significant correlations of this kind currently known are the correlation of the frequency of the *G3PDH-2*e* allele with latitude in the Bering race ($r = 0.497$, $n = 19$, $P < 0.05$) and the correlation of *G3PDH-1*a* with elevation in the Nahanni race ($r = 0.615$, $n = 11$, $P < 0.05$; Foote 1980). Finally, note that within races, no allele frequencies are correlated with longitude, the major axis on which the races are separated.

The formation of a Nahanni race of lake whitefish by introgression of the Bering and Mississippi–Missouri glacial refuge races also appears implausible. It may be seen (Fig. 2) that the Bering race alleles *sIDHP-3*c*, *sMDH-A-1,2*b*, and *mMDH-3*b*, together with the Mississippi–Missouri race alleles *G3PDH-1*b* and *sIDHP-4*c*, are all virtually absent from the widely distributed Nahanni race populations whereas all might be expected to be present if the Nahanni race was the product of introgression of the other races. Indeed, the present results point to very limited introgression among the races (see later).

Dispersal of Lake Whitefish from Glacial Refugia

Dispersal routes of lake whitefish from the Bering and Mississippi–Missouri refugia have been described in detail (Lindsey and McPhail 1986; Franzin and Clayton 1977; Bodaly and Lindsey 1977; Lindsey et al. 1970). Fish dispersing from a Nahanni refugium appear to have first occupied lakes that bordered the previous refugium (21–24) and then dispersed southward to occupy other lakes of the lower (19, 20) and upper (16, 17) Liard watershed as water levels and ecological factors become suitable. The occurrence of Nahanni whitefish in two upper Liard lakes (16, 17; albeit in hybrid forms) indicates that the rapids of the Liard Canyon were either more navigable to lake whitefish immediately following deglaciation than they are

at present or that watershed connections may have previously existed such that the rapids could have been bypassed.

Nahanni race lake whitefish may have continued their dispersal southward into central British Columbia via routes that existed linking the lower Liard River and its Fort Nelson River tributary to Glacial Lake Peace. During deglaciation the outflow of Glacial Lake Peace was shunted north into the Fort Nelson River system when the original outflow towards Glacial Lake Agassiz was blocked by ice (Prest 1970; Mathews 1980; Rampton 1987). Movement of lake whitefish into the upper Fraser system would then have been possible through streams that connected Glacial Lake Peace with Glacial Lake Prince George which drained water from the upper Fraser basin. Such a route would account for how Nahanni whitefish bypassed the navigation barrier posed by the rapids of the Peace River Canyon. Alternatively, lake whitefish may have dispersed southward from the Nahanni glacial refuge during the Wisconsin ice age, or early in deglaciation, via an ice-free corridor between the Laurentide and Cordilleran ice sheets (see Reeves 1973, 1983) which could account for their occurrence in Talbot Lake (33). However, the existence of such a corridor, and hence the plausibility of such a route, remains a subject of considerable debate (White et al. 1985; Lindsey and McPhail 1986).

Introgression of Lake Whitefish Races

There appears to have been little introgression among the Bering, Mississippi–Missouri, and Nahanni glacial refuge lake whitefish races. Only eight populations examined display a combination of alleles indicative of introgression between racial groups. Within these populations, genotype frequencies at all loci examined did not deviate significantly from Hardy–Weinberg equilibrium, and individual fish were found to have alleles distinctive of more than one race (Foote 1980), indicating true introgression and not simply the sympatric occurrence of two or more races.

In the Liard River drainage, there are two narrow zones of introgression among lake whitefish races. Immediately upstream of the rapids of the Liard Canyon, the Toobally and Crooked Lake populations (16, 17) show traits of both the Bering race, which occupy the upper Liard headwater lakes, and the Nahanni race, which occupy the lakes of the lower Liard drainage (e.g. Nahanni race: *LDH-B-2*b* and *sMDH-B-1,2*b* alleles; Bering race: *sMDH-A-1,2*b* and *mMDH-3*b* alleles).

Immediately below the rapids, in the Liard River proper (18) and indeed throughout Mackenzie River proper (39–42), lake whitefish populations displayed traits of both the Bering and Mississippi–Missouri races, indicative of extensive introgression (e.g. Mississippi–Missouri race: *G3PDH-1*b* and *sMDH-B-1,2*b* alleles; Bering race: *mMDH-3*b* allele). Note that the Nahanni race would not be evident in such a combination (i.e. there is no single allele distinctive of the Nahanni race). However, it seems likely that this race has also introgressed with other racial forms directly in the river given the preponderance of Nahanni race populations in the region.

Cox Lake (43) lake whitefish of the Richardson River system on the Arctic coast also displayed a combination of Bering and Mississippi–Missouri race alleles. The presence of Bering alleles in Cox Lake lake whitefish suggests coastal dispersal by Bering lake whitefish, and hence three Bering lake whitefish entry points into the Mackenzie River system: the upper Liard (Franzin and Clayton 1977), the Peel (Bodaly and Lindsey 1977), and the Mackenzie estuary (see Lindsey and McPhail 1986).

Finally, there is further evidence from three lake whitefish populations in Alberta (28, 35, 37) of introgression between the Nahanni and Mississippi-Missouri races. These populations, situated between Nahanni race populations to the west and Mississippi-Missouri to the east, show intermediate frequencies of *G3PDH-1*b*, an allele which is absent in the Nahanni race but present in the Mississippi-Missouri race.

Physical barriers to dispersal have undoubtedly limited the opportunity for introgression among races of lake whitefish (Franzin and Clayton 1977) but are not solely accountable for the limited introgression observed in this study. We have found both "pure" Nahanni and "pure" Mississippi-Missouri populations to persist in the absence of such barriers. For example, Bovie (20) and Fisherman (19) lakes, tributary to the mainstream of the lower Liard River, and Little Doctor Lake (24), tributary to the Mackenzie River, all have short navigable outlets to the major rivers, have fish species compositions indicative of invasion by Mississippi-Missouri species (e.g. walleye, *Stizostedion vitreum*, Foote 1980), but yet, non-Nahanni race *G3PDH-1*b*, *G3PDH-2*e*, *sIDHP-3*c*, and *mMDH-3*b* alleles present in the nearby river populations (18, 39) are completely absent from these three lake populations. A total of 77 fish were sampled from the three lakes, representing a 616-allele sample for the four loci combined ($77 \times 2 \times 4$). Similarly, we found none of the three alleles distinctive of the Bering race (*sIDHP-3*c*, *sMDH-A-1,2*b*, and *mMDH-3*b*) in our 250-fish sample (1500 alleles) of what turned out to be "pure" Mississippi-Missouri race lake whitefish in Great Slave Lake (38). In marked contrast, we found a mixture of Bering and Mississippi-Missouri alleles in an 11-fish sample taken from the Mackenzie River at Fort Simpson (39), some 300 easily navigable kilometres downstream from the lake outlet.

The reasons for the extremely limited introgression among lake whitefish races in the lakes, but not rivers, of the Mackenzie River system remain unclear. Perhaps the races are able to competitively exclude each other in lacustrine but not riverine environments. Whatever the case, we know of no example where different races occur sympatrically and remain genetically distinct. This observation contrasts markedly with the existence of sympatric, morphologically, ecologically, and genetically distinct forms of lake whitefish within the glacial refuge races (Bodaly et al. 1992; Bernatchez and Dodson 1990).

In conclusion, we have presented biological evidence for the existence of a third, Nahanni glacial refuge race of lake whitefish in western Canada. Geological evidence, coupled with genetic characteristics and the present distribution of populations of this race, indicates that the Nahanni glacial refuge race of lake whitefish may have diverged genetically from the Bering and Mississippi-Missouri races while in isolation in a Nahanni glacial refugium.

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(Appendix 1 follows on the next page)

TABLE A.1. Frequencies of alleles at polymorphic loci and "fast" hemoglobin phenotype for 43 lake whitefish populations examined. Location number corresponds to numbers given in Fig. 1. Allele mobilities are given in Table 2. The proposed racial origin of each population is identified: B = Bering refuge race; N = Nahanni refuge race; M = Mississippi-Missouri "hybrid" populations. Source: A = this study; B = Franzin and Clayton (1977); C = Bodaly et al. (1992); D = Bodaly and Lindsey (1977).

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Evolution of Lake Whitefish (*Coregonus clupeaformis*) in North America during the Pleistocene: Genetic Differentiation between Sympatric Populations¹

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We use electrophoretic data on dehydrogenase enzymes to examine the relatedness of sympatric populations of lake whitefish (*Coregonus clupeaformis*) and provide evidence for the existence of a glacial refuge race of lake whitefish in eastern North America. This Acadian race is presently found in New England, the Gaspé peninsula of Québec, and New Brunswick. It probably survived glaciation in a refugium on the exposed coastal plain of northeastern North America. In areas of contact, most glacial races appear to introgress and do not coexist in sympatry. However, sympatric pairs of populations occur (or occurred) within the ranges of all races of lake whitefish. Allele frequencies for at least one enzyme system examined for most sympatric pairs were significantly different, indicating that these sympatric populations are wholly or substantially isolated reproductively from each other. Both members of the population pairs examined in the Yukon Territory, Ontario, and Labrador were genetically characteristic of the glacial races of their region. This suggests that they are not the result of speciation due to geographic isolation in different glacial refugia. Thus, their origin appears to be postglacial, but may be older if present genetic similarities are due to recent gene flow between sympatric forms.

Nous avons utilisé des données électrophorétiques sur les enzymes déshydrogénases pour étudier le caractère de parenté de populations de Grand corégone (*Coregonus clupeaformis*) sympatriques et obtenir des indices de l'existence d'une race de refuge glaciaire dans l'est de l'Amérique du Nord. Cette race acadienne se rencontre actuellement en Nouvelle-Angleterre, en Gaspésie (Québec) et au Nouveau-Brunswick. Cette espèce a probablement survécu à la glaciation dans un refuge situé dans la plaine côtière exposée du nord-est de l'Amérique du Nord. Dans les zones de contact, il semble y avoir eu introgression de la plupart des races glaciaires qui n'étaient pas sympatriques. Des paires de populations sympatriques existent cependant (ou ont existé) au sein des aires de répartition de toutes les races de Grand corégone. Les fréquences alléliques d'au moins un des systèmes enzymatiques étudiés pour la majorité des paires sympatriques différaient de façon significative, ce qui montre que ces populations sympatriques ont des reproductions totalement ou fortement isolées les unes des autres. Les deux membres des paires de population examinées du territoire du Yukon, de l'Ontario et du Labrador présentaient les caractéristiques génétiques des races glaciaires de leur région. Cela porte à croire qu'elles ne résultent pas d'une spéciation par isolement géographique dans des refuges glaciaires distincts. Elles semblent donc avoir une origine post-glaciaire, mais elles pourraient être plus vieilles si les similitudes génétiques actuelles s'expliquaient par un flux génétique entre formes sympatriques.

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Coregonid fishes have undergone extensive recent speciation. Species flocks of ciscoes occur in the Laurentian Great Lakes (Smith and Todd 1984) and sympatric forms are known from many of the species in the group (e.g. pygmy

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whitefish (*Prosopium coulteri*) (McCart 1970), cisco (*Coregonus artedii*) (Clarke 1973), vendace (*C. albula*) (Vuorinen et al. 1981), and European whitefish (*C. lavaretus*) (Svärdson 1970). Various modes of speciation have been hypothesized to account for sympatric populations of coregonids, including geographic speciation due to isolation in different glacial refugia (McCart 1970; Svärdson 1970), sympatric speciation within individual lakes (Steinmann 1951), and microgeographic speciation during deglaciation (Behnke 1972). Sympatric coregonid forms have been identified by organismal characteristics, but protein electrophoresis has been employed in recent studies

TABLE 1. List of enzymes studied, their enzyme numbers, abbreviations used, number of loci, and tissues examined (M = muscle; L = liver; H = heart).

Enzyme	Enzyme Commission No.	Abbreviation	No. of loci	Tissue
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	2	M
Isocitrate dehydrogenase	1.1.1.42	sIDHP	2	L
Lactate dehydrogenase	1.1.1.27	LDH-A	2	M
		LDH-B	2	H
Malate dehydrogenase	1.1.1.37	sMDH-A	2	L
		sMDH-B	2	M
		mMDH	3	M

to attempt to elucidate phylogenetic relationships. For example, Vuorinen et al. (1981) examined populations of the vendace in Finland, including sympatric forms. Genetic characters seem to be well suited for these types of studies because of the decreased possibility that similarities between populations can be the result of convergent or parallel evolution as is the case with morphological and behavioral characteristics.

The lake whitefish (*Coregonus clupeaformis*) species complex occurs throughout most of the northern half of North America, including Alaska, most of Canada, and parts of New England (Lindsey et al. 1970). It has been shown to be composed of probably five geographic races or subspecies which diverged as a result of isolation in different glacial refugia (Lindsey et al. 1970; Franzin and Clayton 1977; Bernatchez and Dodson 1991; Foote et al. 1992). Within each of these races there exists (or existed) at least one instance of sympatric pairs of forms in individual lakes (Lindsey et al. 1970; Bodaly 1979; Bruce 1984; Kirkpatrick and Selander 1979; Fortin and Gendron 1990; Bodaly et al. 1991a). Lindsey et al. (1970) showed that, on the basis of three protein systems examined, one of the sympatric pairs in the Yukon Territory, one in Ontario, and one in Maine did not share a common origin. This paper extends these observations by increasing the number of genetic loci and the number of sympatric pairs examined, including recently discovered sympatric pairs in Labrador and Ontario, and three additional pairs in the Yukon. Isozyme evidence for the existence of the Acadian glacial refuge race from northern New England and adjacent areas of Canada is described and the amount of genetic differentiation between the glacial refuge races and sympatric pairs is compared.

Methods and Materials

One hundred and nineteen lake whitefish populations from across North America were sampled over the period 1968–86. These included both members of the sympatric pairs of lake whitefish forms from Dezadeash, Squanga, Little Teslin, and Teenah lakes (Yukon Territory) (high and low gill raker forms), Como Lake (Ontario), Ossokmanuan Reservoir (Labrador), and Cliff Lake (Maine) (dwarf and normal forms). (In addition, allele frequencies at the *LDH-B-2** locus from Kirkpatrick and Selander (1979) for the sympatric pair found in Musquacook Lake, Maine, were included in the results.) The normal size morph only of the sympatric pair of forms from Opeongo Lake (Ontario) was also examined. Fish were obtained by gillnetting, frozen soon after capture, and stored frozen at -40°C for varying periods until analysis. The enzyme protein products of 15 genetic loci for four dehydrogenase enzyme systems (glycerol-

3-phosphate, isocitrate, lactate, and malate dehydrogenase) were examined by starch gel electrophoresis (Table 1). Not all enzyme systems were examined for all populations. Sample sizes of all populations for individual enzymes ranged from 2 to 518 and averaged 46. Details of methods, genetic models, and allele mobilities are found in Foote et al. (1992). The system of nomenclature for isozymes, loci, and alleles follows Shaklee et al. (1989). Published data from Casselman et al. (1981), Foote et al. (1992), Franzin and Clayton (1977), Ihssen et al. (1981), Imhof et al. (1980), Kirkpatrick and Selander (1979), and Lindsey et al. (1970) were also utilized in this investigation.

Results

*LDH-B-1** and *LDH-B-2** Loci

All fish examined were fixed for the same allele at the *LDH-B-1** locus. At the *LDH-B-2** locus, populations over most of the range of the lake whitefish showed high frequencies (usually >0.75) of the *LDH-B-2*b* allele and low frequencies of the *LDH-B-2*a* allele (Fig. 1a). In the Yukon Territory and northern British Columbia, however, the *LDH-B-2*b* allele was not found. The *LDH-B-2*d* allele was found only in the Grand Lake, New Brunswick, population. Sympatric pairs of populations were all typical of their geographic area, including four pairs in Yukon, two in Maine, one in Labrador, and one pair and the single member of the Opeongo Lake pair in Ontario.

*LDH-A-1** and *LDH-A-2** Loci

All fish were fixed for the same allele at the *LDH-A-1** locus. At the *LDH-A-2** locus, the *LDH-A-2*a* allele predominated in all populations, with frequencies ranging from 0.83 to 1.0 and the mode at 1.0. The *LDH-A-2*c* allele was present in many populations, in frequencies ranging from 0 to 0.17. The *LDH-A-2*b* allele was found only in the Talbot Lake, Alberta, population at a frequency of approximately 0.1. There were no discernible geographic patterns in the frequency of alleles at this locus for the 43 populations examined, and therefore the results are not shown.

*G3PDH-1** Locus

Populations in the central part of the range of lake whitefish were characterized by moderate frequencies of the *G3PDH-1*a*, *G3PDH-1*b*, and *G3PDH-1*d* alleles (Fig. 1b). Populations in the Yukon Territory, the southwest corner of the Northwest Territories, and all of British Columbia lacked the *G3PDH-1*b* allele. The *G3PDH-1*a* allele, which was the only allele found in the Labrador sympatric pair, was absent in populations in Maine and New Brunswick. One member of a Maine sympatric pair was the only population known with the *G3PDH-1*g* allele. The rare, electrophoretically slow *G3PDH-1*h* allele was found only in one population in southeastern Yukon Territory at a frequency too low to be shown on Fig. 1b. *G3PDH-1** allele frequencies for sympatric pairs in the Yukon Territory and Ontario were typical of their geographic area. Although both members of the Como Lake sympatric pair lacked the *G3PDH-1*d* allele, this was not due to small sample sizes because 50 fish of each form were analyzed. Both members of the Labrador pair were fixed for the *G3PDH-1*a* allele. The *G3PDH-1*g* allele was present in one member of a Maine sympatric pair.



FIG. 1. Distribution of alleles at polymorphic loci which show geographic patterns in lake whitefish populations. Sympatric pairs of lake whitefish populations are identified by letter codes as follows: D = Dezadeash Lake; S = Squanga Lake; LT = Little Teslin Lake; T = Teenah Lake; CO = Como Lake; OP = Opeongo Lake; OS = Ossokmanuan Reservoir; CL = Cliff Lake; M = Musquacook Lake. Data from Casselman et al. (1981), Foote et al. (1992), Franzin and Clayton (1977), Ihssen et al. (1981), Imhof et al. (1980), Kirkpatrick and Selander (1979), and Lindsey et al. (1970) are included in this figure. (Fig. 1 continued next page)

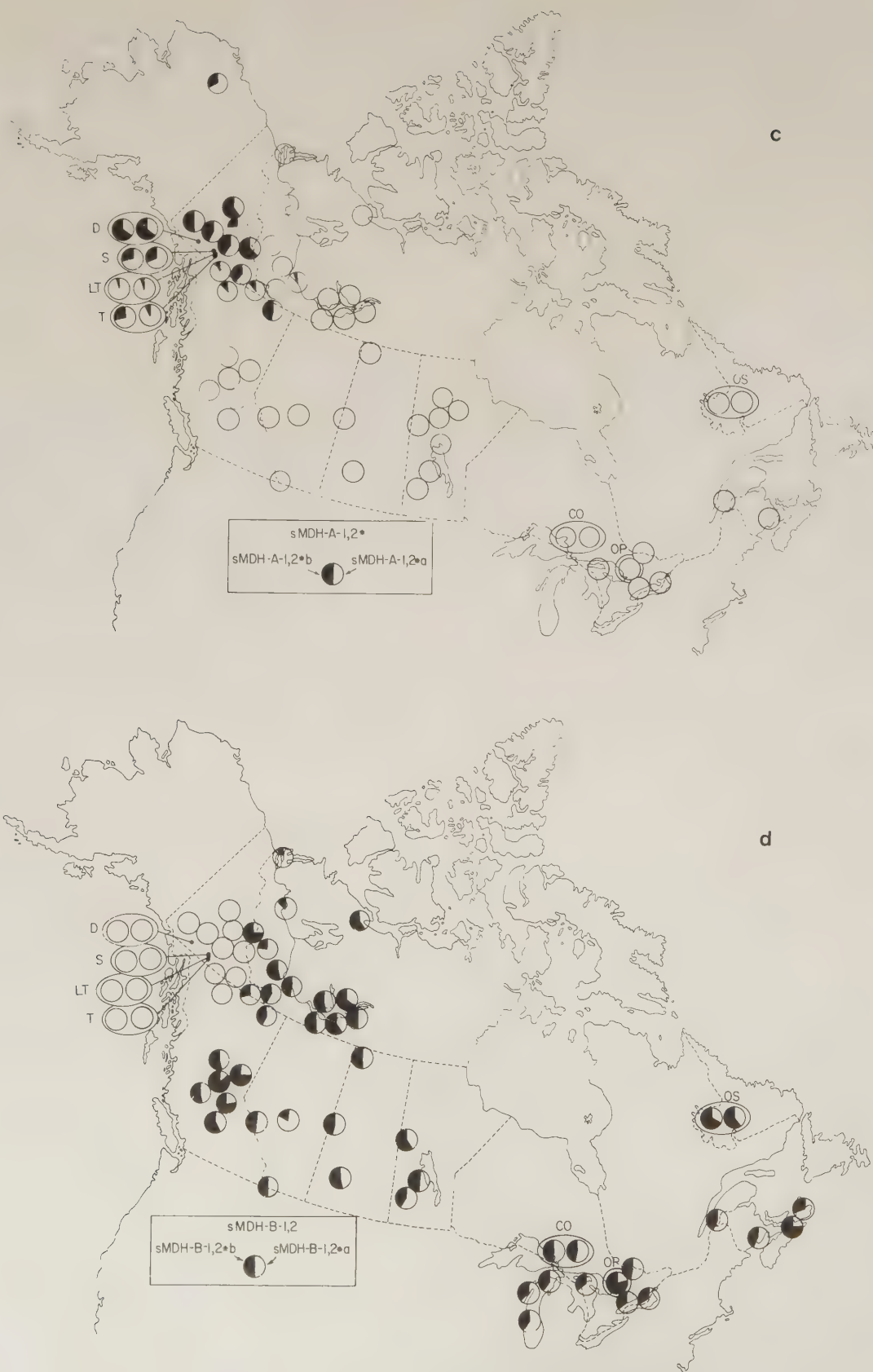


FIG. 1. (Continued)

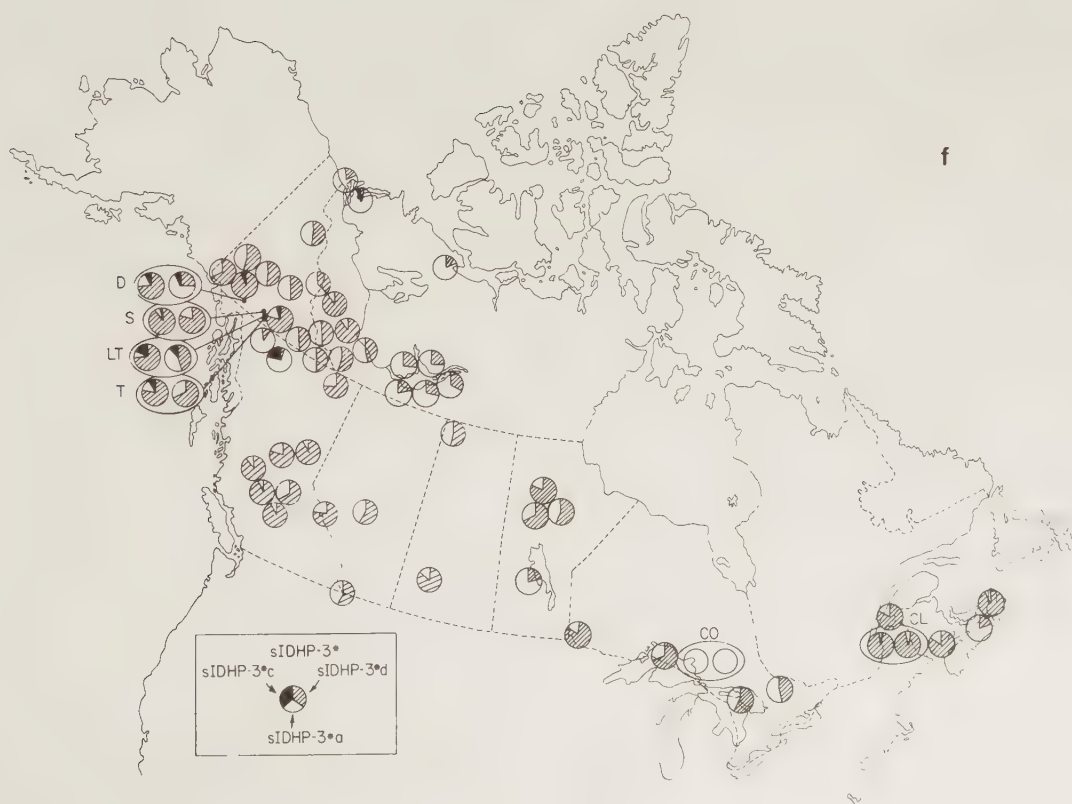


FIG. 1. (Continued)

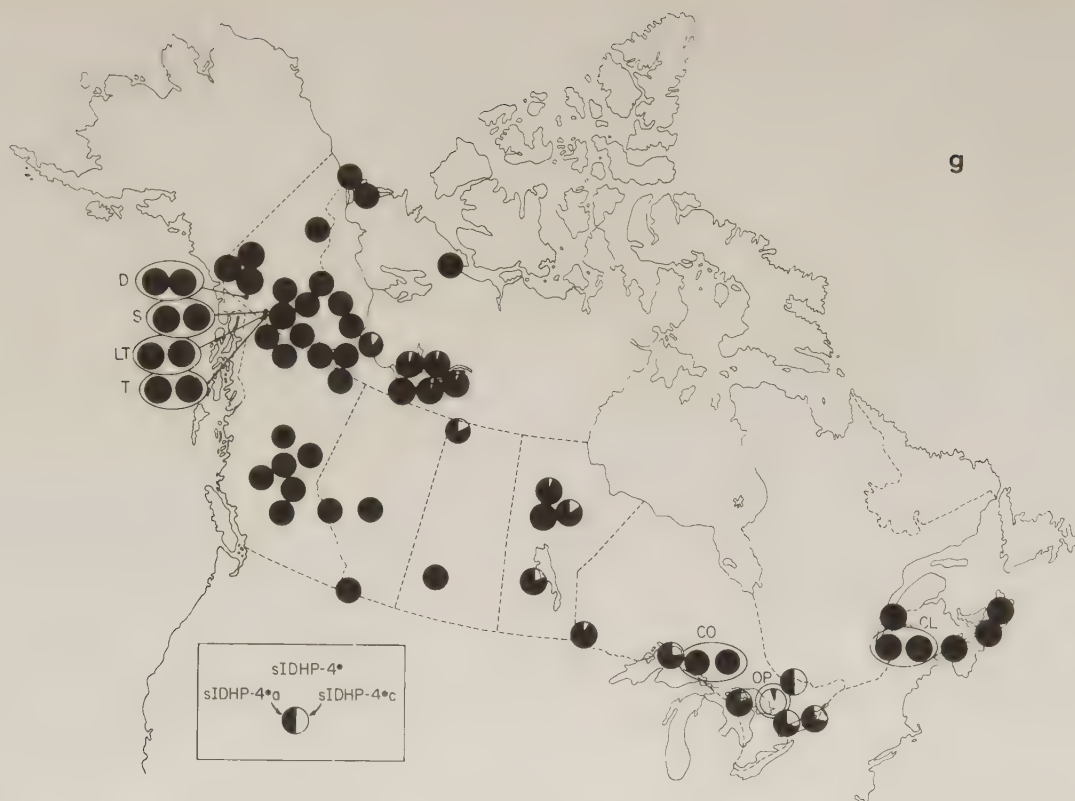


FIG. 1. (Concluded)

*G3PDH-2** Locus

The *G3PDH-2*a* allele predominated in most populations, with frequencies ranging from 0.46 to 1.0 and the mode at 1.0 (not presented in a figure). There was no discernable geographic pattern of allele frequencies at this locus.

*sMDH-A-1,2** Loci

Populations in the central and eastern parts of the range of lake whitefish as well as those in British Columbia were fixed for the *sMDH-A-1,2*a* allele (Fig. 1c) whereas Yukon Territory populations exhibited low to moderate frequencies of the *sMDH-A-1,2*b* allele. Sympatric pairs in the Yukon Territory, Ontario, and Labrador were typical of their geographic area.

*sMDH-B-1,2** Loci

Lake whitefish populations from British Columbia east to Maine, New Brunswick, and Labrador were characterized by moderate to high frequencies of the *sMDH-B-1,2*b* allele whereas this allele was absent from all Yukon Territory populations (Fig. 1d). Sympatric pairs in the Yukon Territory, Ontario, and Labrador were all typical of their geographic area.

mMDH Loci

All fish were fixed for the same alleles at the *mMDH-1** and *mMDH-2** loci, but variation was seen at the duplicated *mMDH-3** locus. Throughout the central part of the range of the lake whitefish, from British Columbia to Maine, New Brunswick, and Labrador, populations were characterized by fixation of the *mMDH-3*a* allele (Fig. 1e). Many, but not all, Yukon populations showed moderate frequencies of the *mMDH-3*b* allele. Both members of one of the four Yukon

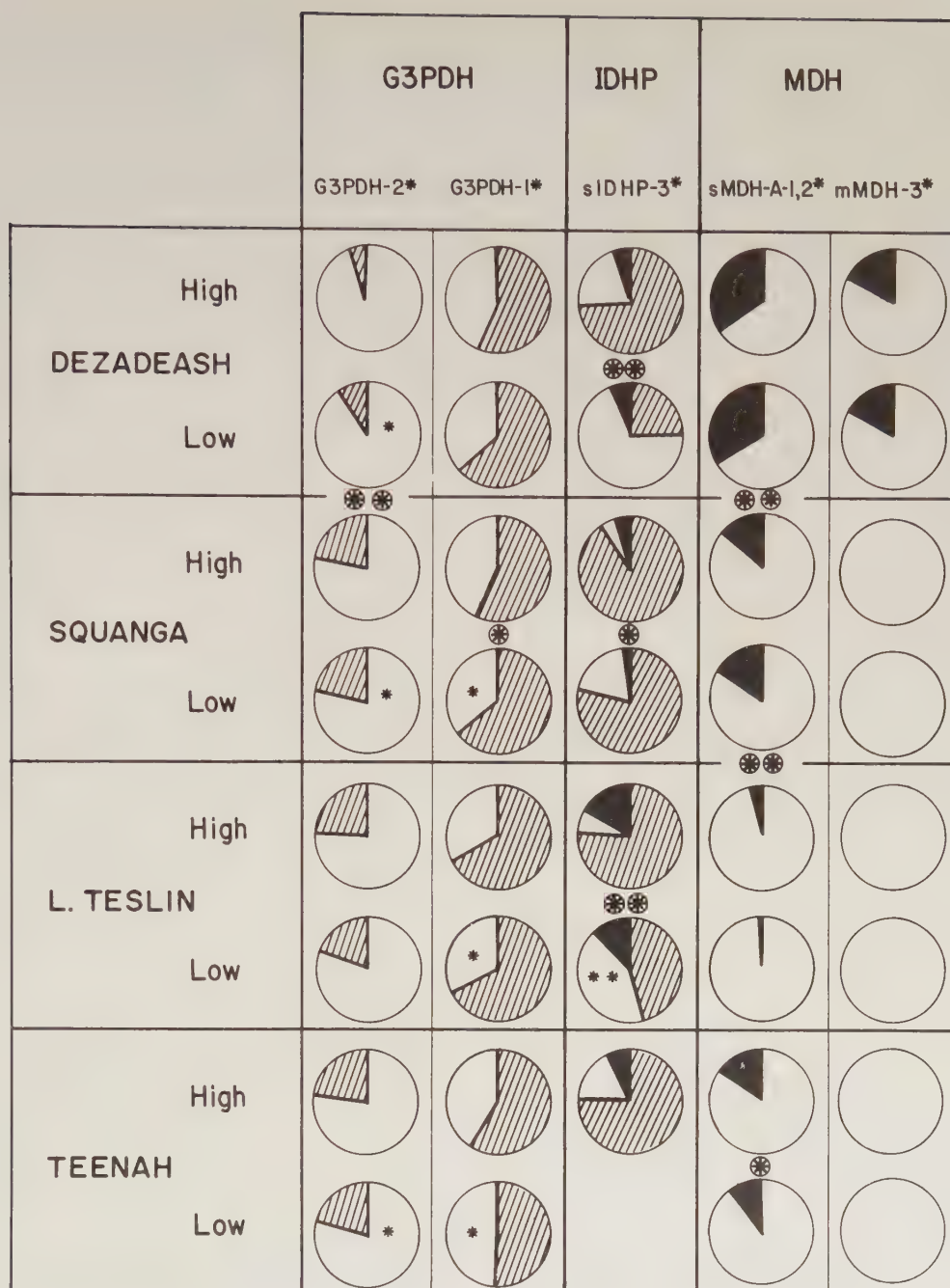
Territory sympatric pairs exhibited moderate frequencies of the *mMDH-3*b* allele whereas the other three Yukon pairs were fixed for the *mMDH-3*a* allele. The Labrador sympatric pair, the pair in Como Lake, and one Opeongo Lake population were typical of their geographic areas.

*sIDHP-3** Locus

Populations in the central part of the range of lake whitefish were characterized by moderate frequencies of the *sIDHP-3*d* allele (Fig. 1f). The *sIDHP-3*c* allele was found only in Yukon Territory populations, being present in about half of the populations sampled (often in frequencies too small to show in Fig. 1f). The frequency of the *sIDHP-3*a* allele was much lower in Maine and New Brunswick populations than in all other populations examined. Yukon sympatric pairs were found to be typical of their geographic area, although most contained the *sIDHP-3*c* allele, as was the one sympatric pair of populations in Maine. In common with all other populations in the central part of the range of lake whitefish, the Como Lake, Ontario, pair was lacking the *sIDHP-3*a* allele, but it was also lacking the *sIDHP-3*d* allele.

*sIDHP-4** Locus

Lake whitefish populations in the Yukon Territory and British Columbia and in Maine and New Brunswick were generally fixed for the *sIDHP-4*a* allele whereas the *sIDHP-4*c* allele was found in low to moderate frequencies in populations in the center of the range of the species (Fig. 1g). Exceptions to this pattern were the Little Teslin Lake (Y.T.) high gill raker population which had the *sIDHP-4*c* allele in low frequency (seen in only one fish) and both forms in Teenah Lake (Y.T.) which had a rare fast allele in very low frequency (seen in only one



*, ** INDICATES DEPARTURE FROM C-H-W EXPECTED PHENOTYPE DISTRIBUTION
 * AT $P < 0.05$; ** AT $P < 0.001$

⊗, ⊗⊗ INDICATES DEPARTURE FROM ALLELE NUMBER EXPECTED DISTRIBUTION
 ⊗ AT $P < 0.05$; ⊗⊗ AT $P < 0.001$

FIG. 2. Allele frequencies at polymorphic loci for sympatric populations of lake whitefish in four Yukon Territory lakes. High = high gill raker form; Low = low gill raker form. Allele designations are the same as in Fig. 1. Asterisks indicate departures from Castle-Hardy-Weinberg expected phenotype distributions.

fish of each form). Crooked Lake (B.C.) lake whitefish had both the *sIDHP-4*c* and *sIDHP-4*d* alleles in low frequencies.

Most Yukon sympatric populations were in Hardy-Weinberg equilibria, except for many low gill raker populations for the two G3PDH loci (Fig. 2). This observation is unexplained, but is not due to a fault in the genetic model, which is based on breeding experiments (Clayton et al. 1973). Also, these loci did not show departure from Hardy-Weinberg equilibria in

most of the other populations which were examined. There were significant allele frequency differences between high and low gill raker populations at at least one locus for every one of the sympatric pairs (Fig. 2). In Dezadeash, Squanga, and Little Teslin lakes, the high raker form was significantly different from the low raker form in the frequency of alleles at the *sIDHP-3** locus. In Squanga Lake, the sympatric populations differed also in the frequency of *G3PDH-1** locus alleles. In Teenah

TABLE 2. Genetic distances (Nei 1978) based on 15 loci between selected populations of lake whitefish.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Bering race														
1. Dezadeash L., Yukon (high raker)														
2. Dezadeash L., Yukon (low raker)	0.018													
3. Little Teslin L., Yukon (high raker)	0.020	0.044												
4. Little Teslin L., Yukon (low raker)	0.029	0.027	0.007											
5. Squanga L., Yukon (high raker)	0.030	0.031	0.018	0.015										
6. Squanga L., Yukon (low raker)	0.010	0.030	0.002	0.009	0.020									
Nahanni race														
7. Cache L., N.W.T.	0.208	0.205	0.197	0.186	0.194	0.202								
8. Fisherman L., N.W.T.	0.140	0.145	0.122	0.117	0.133	0.126	0.033							
9. McLeod L., B.C.	0.132	0.157	0.114	0.120	0.134	0.120	0.023	0.005						
10. Seaplane L., N.W.T.	0.134	0.174	0.122	0.136	0.143	0.128	0.063	0.026	0.018					
Mississippi race														
11. Diefenbaker L., Sask.	0.130	0.160	0.106	0.115	0.137	0.111	0.047	0.014	0.007	0.043				
12. Great Slave L., N.W.T. (Yellowknife)	0.189	0.147	0.169	0.136	0.159	0.170	0.052	0.034	0.055	0.116	0.050			
13. Como L., Ont. (dwarf)	0.214	0.173	0.206	0.172	0.182	0.207	0.033	0.033	0.051	0.079	0.074	0.021		
14. Como L., Ont. (normal)	0.207	0.166	0.199	0.165	0.175	0.200	0.036	0.033	0.051	0.075	0.075	0.023	0.000	

Lake, the sympatric populations differed in the frequency of alleles at the *sMDH-A-1,2** loci.

The high and low gill raker populations in Dezadeash Lake were genetically different from populations in the three Squanga Creek lakes (Squanga, Teenah, and Little Teslin) (Fig. 2). Both Dezadeash populations showed an appreciable frequency of the *mMDH-3*b* allele whereas this allele was absent from all of the Squanga Creek populations. Also, the Dezadeash pair had allele frequencies at the *sMDH-A-1,2* loci and at both G3PDH loci, which were significantly different from the Squanga Creek populations.

Genetic divergence among populations in different regions representing different glacial refuge races was much greater than between sympatric populations (Table 2). For instance, the average Nei (1978) genetic distances among populations representing the Bering, Nahanni, and Mississippi-Missouri glacial refuge races (number of populations compared in parentheses) were as follows:

	Nahanni	Mississippi
Bering	0.15 (24)	0.17 (24)
Nahanni		0.05 (16)

Bodaly et al. (1991b) found an average genetic distance between Mississippi and Bering race lake whitefish of 0.10. This estimate was based on the products of 37 genetic loci. The mean genetic distance between sympatric populations in Yukon lakes was much less, only 0.015 (Table 2), and the average genetic distance between the sympatric populations in Como Lake, Ontario, was <0.01.

Discussion

Lake Whitefish Races in North America

Major allozyme differences between lake whitefish inhabiting the Yukon Territory and those in the rest of western Canada were identified by Lindsey et al. (1970) and Franzin and Clayton (1977). These data were interpreted as evidence for the existence of two races of lake whitefish, the Bering glacial refuge race and the Mississippi-Missouri glacial refuge race that were descended from fish that survived Wisconsinan glaciation in the Bering and Mississippi-Missouri refugia, respectively. Foote et al. (1992) demonstrated the existence of a third Nahanni glacial refuge race, in British Columbia and southwestern Northwest Territories. It was suggested that this race survived Wisconsinan glaciation in a Nahanni glacial refugium in the vicinity of present-day Nahanni National Park in the Northwest Territories.

The survival of lake whitefish during Wisconsinan glaciation in the northern part of the Atlantic slope of the Appalachians was suggested by Lindsey et al. (1970) based on the present distribution of the species and by the occurrence of a few populations with quite high gill raker counts in the area of the Laurentian Great Lakes and the drainages of the St. Lawrence River. Bernatchez and Dodson (1990, 1991) examined mtDNA variation in lake whitefish populations across North America and concluded that three glacial refuge races of lake whitefish exist in eastern North America. The Acadian race was found to be present in lakes of New Brunswick, Nova Scotia, Maine, and the Gaspé peninsula of Québec. It was presumed to be descended from fish that survived the Wisconsinan glaciation in the Northeastern Banks refugium (Schmidt 1986). The

Atlantic race, now found in Maine and southern Québec, was presumed to have survived in the Atlantic glacial refugium (Underhill 1986). The third lake whitefish race identified in eastern North America by Bernatchez and Dodson (1991) was the Mississippi–Missouri race. It was present to the west and north of the Acadian and Atlantic races.

The distribution of alleles at all 11 polymorphic loci in the present work provides additional evidence for the existence of at least four genetically and geographically definable races of lake whitefish in North America. The Bering glacial refuge race is found in the central and southern Yukon Territory, except for the lower Liard basin, and in northwestern British Columbia. The Bering race, compared with the Mississippi–Missouri race, is characterized by missing alleles at the *LDH-B-2**, *G3PDH-1**, *sMDH-B-1,2**, and *sIDHP-4** loci and additional alleles at the *sMDH-A-1,2**, *mMDH-3**, and *sIDHP-3** loci. The Mississippi–Missouri race occupies much of the central part of the range of the lake whitefish, including Alberta, Saskatchewan, Manitoba, Ontario, Québec, and Labrador and much of the Northwest Territories. The Nahanni glacial refuge race is found in British Columbia, in the southwest corner of the Northwest Territories, and in the southeast corner of the Yukon Territory (Foote et al. 1992). The Nahanni race is distinguished from the Mississippi–Missouri race by missing alleles at the *G3PDH-1** and *sIDHP-4** loci and an additional allele at the *G3PDH-1** locus. A fourth lake whitefish race, the Acadian race, found in Maine, New Brunswick, Nova Scotia, and the Gaspé, differs from the Mississippi–Missouri race by missing alleles at the *sIDHP-4** and *G3PDH-1** loci, by having very different allele frequencies at one other locus (*sIDHP-3**), and by the existence of unique alleles in two populations. Readers are referred to Foote et al. (1992) and Bernatchez and Dodson (1991) for maps showing the geographic extent of these races.

Allozyme evidence presented here is consistent with the conclusions of Bernatchez and Dodson (1991), based on mtDNA, regarding the existence of an Acadian race. However, allozyme results do not suggest the existence of an Atlantic race, as distinct from the Mississippi–Missouri race. Because the mtDNA results appear to provide finer resolution of geographic patterns in the lake whitefish, the allozyme results do not refute the conclusions based on mtDNA. Also, the allozyme genetic differences between the Acadian and adjacent races were insufficient to permit detection of the area of overlap in Maine because of the large amount of interpopulation variation present and because the two distinctive alleles present in the Atlantic race were found in only two populations.

Introgression between Glacial Refuge Races

There is no evidence for sympatric populations that could be considered to reflect reproductive isolation where the Nahanni, Bering, or Mississippi–Missouri geographic races of lake whitefish have come together. In some instances, geographic barriers have limited the mixture of alleles between glacial refuge races of lake whitefish. For example, the Liard Canyon has prevented the flow of Nahanni race alleles into the Bering race (Foote et al. 1992). Similarly, Mississippi–Missouri race alleles have apparently not moved up the Peel River due to the present unsuitability of the physical environment or physical barriers (Bodaly and Lindsey 1977). Whereas there is no evidence for the sympatric coexistence of two or more lake whitefish glacial refuge races, there are many areas where different races of lake whitefish have introgressed (Foote et al. 1992). Introgression is evident in the Mackenzie Delta (Bering and

Mississippi–Missouri race) (Bodaly and Lindsey 1977) and at the confluence of the Liard and Mackenzie rivers at Fort Simpson (Nahanni and Mississippi–Missouri races) (Foote et al. 1992). In Alberta, lake whitefish populations may be intergrades between the Nahanni and Mississippi–Missouri races. This is suggested by the distribution of alleles at the *G3PDH-1** and *sIDHP-4** loci (Fig. 1b and 1g).

Divergence between Sympatric Forms

Bernatchez and Dodson (1990) have interpreted their mtDNA data as evidence that sympatric pairs of lake whitefish in lakes in Maine reflect continued reproductive isolation of the Mississippi–Missouri (Atlantic) and Acadian races in sympatry after allopatric divergence. This interpretation is in contrast with the present results and the results of Lindsey et al. (1970) and Foote et al. (1992), which suggest that the sympatric forms in the Bering and Mississippi–Missouri races have arisen within the respective races and provide no evidence for any biological barriers to introgression of the lake whitefish races. Zoogeographic evidence suggests that the process of divergence which resulted in the sympatric forms in Maine was different from the process of divergence which has produced sympatric pairs in the Yukon Territory, Ontario, and Labrador. In Maine, sympatric pairs are known from at least 22 lakes whereas only 10 instances of sympatric pairs are known over the whole remainder of the range of the species. The known genetic differences between the Mississippi–Missouri (Atlantic) and Acadian races are much less than those between the Mississippi–Missouri and Bering races (Fig. 1), and it is curious, therefore, that reproductive isolation should have occurred and be maintained in the contact zone between the Atlantic and Acadian races. The amount of genetic divergence between lake whitefish races, as measured by allozyme allele frequencies, is apparently a poor predictor of the tendency for reproductive isolation between those races.

Sympatric pairs have diverged significantly in life history traits and morphology but apparently little genetically (at least in the sample of enzyme coding loci utilized in this study) whereas geographic races have diverged to a much greater degree genetically but apparently little in life history or morphology. Sympatric pairs differ in morphology (especially of the gill raker apparatus), feeding traits, growth, and age at maturity (Kennedy 1943; Bodaly 1979; Bodaly et al. 1991a; Fenderson 1964; Kirkpatrick and Selander 1979; Bruce 1984; Fortin and Gendron 1990). Also, sympatric populations are completely or substantially isolated reproductively. Most exhibit significantly different protein allele frequencies (Fig. 2) (Bodaly et al. 1988; Kirkpatrick and Selander 1979), and where spawning aggregations have been sampled, sympatric forms have usually been shown to spawn at different times and/or places (Lindsey 1963; Fenderson 1964; Kennedy 1943; Kirkpatrick and Selander 1979). The Como Lake, Ontario, sympatric pair do not show differences in allele frequencies, but there are indications of differences in the frequencies of different mtDNA clones in the two forms (L. Bernatchez, University of Guelph, unpubl. data). Therefore, sympatric lake whitefish forms are not cases of polymorphisms within single populations as has been documented in other species (e.g. Sage and Selander 1975). Geographic races, in contrast, have apparently diverged little in ecological and morphological traits, showing only slight differences in modal gill raker counts (Lindsey et al. 1970; Bodaly and Lindsey 1977; Foote et al. 1992). This indicates a lack of correspondence between (pro-

tein) genetic and organismal evolution, as has been noted for other fishes (e.g. Clayton 1981).

If speciation has occurred after the beginning of the retreat of Wisconsinan ice, then it could be the result of genetic reorganizations during colonization of new habitat during deglaciation, or it could be due to sympatric speciation in specific ecological situations. Templeton (1981) has argued, in a review of various mechanisms of speciation, that reproductive isolation resulting from genetic changes during founder events should be relatively rare. Sympatric pairs of lake whitefish populations are indeed rare in the Bering, Mississippi–Missouri, and Nahanni races whereas they are common in the zone of contact between the Mississippi–Missouri (Atlantic) and Acanadian races (Fenderson 1964). It was also argued that sympatric speciation is most likely only when disruptive selection is operative on a trait which itself produces isolating barriers (Templeton 1981). However, it seems possible that rapid evolution could occur during the colonization of waters in recently deglaciated regions and could result in geographic speciation on a short time scale and small geographic scale, as was suggested by Behnke (1972).

The high gill raker form of lake whitefish in Yukon Territory lakes is probably a polyphyletic group. This finding is similar to observations from other studies on sympatric morphs within the Salmonidae. Vuorinen et al. (1981) found that genetic distances between sympatric winter/spring- and autumn-spawning vendace in Finland indicated that the winter/spring spawners had arisen independently more than once from autumn-spawning populations. Similarly, Hindar et al. (1986) found that ecological morphotypes of Arctic char (*Salvelinus alpinus*) in Norway appeared to have arisen independently at a number of locations.

If the genetic similarities between member populations of sympatric pairs in Yukon Territory lakes are the result of post-Wisconsinan gene flow, then their divergence may, in fact, be older than post-Pleistocene. This seems unlikely because the genetic distance between the two forms in Dezadeash, which are the most distinct morphologically and almost certainly have no present gene flow between them (Bodaly 1979), differs little from the genetic distances between the two forms in the three Squanga Creek lakes where it would appear more likely that gene flow is occurring presently or has occurred in the recent past. There is, however, zoogeographic evidence to suggest that the high raker form could have developed in an Alsek River basin subrefuge of the Bering refugium. The Alsek River basin includes the present-day Dezadeash Lake that supports one of the Yukon Territory sympatric pairs. Tempelman-Kluit (1980) has hypothesized that before the Pleistocene, the drainage patterns of the southwestern Yukon Territory were very different from what they are today. Most of the Yukon River basin, including the McQuesten River watershed where Hanson Lake is now located, drained south and west into the Alsek River. At the same time the southernmost present-day Yukon River basin, including the Teslin River and Squanga Creek watersheds, drained to the southeast to the Stikine River. The present drainage patterns in these areas are supposed to have been established before the last of three ice advances in the area, except for disruptions that occurred as a result of ice blockage during recent successive ice advances in the St. Elias Mountains when most of the Alsek River basin became tributary to the Yukon River (Tempelman-Kluit 1980). This isolation of the Alsek River and middle Yukon River basins from the Squanga Creek watershed and from the lower Yukon River basin during the Pliocene and early Pleistocene could have pro-

vided geographic isolation for the development of the high raker form of lake whitefish in the Yukon Territory (Lindsey and McPhail 1986). Studies utilizing other genetic techniques may provide a test of these alternative hypotheses of divergence.

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Use of Dissolved Strontium in Scale Marking of Juvenile Salmonids: Effects of Concentration and Exposure Time

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Strontium (Sr), which is chemically similar to calcium and can substitute for this element in bony tissues of fishes, shows promise as an inexpensive and efficient means of chemically marking juvenile salmonids. This study examines the effects of three different Sr concentrations in water (1.8, 3.6, and 5.4 mg/L) and two different exposure times (30 and 60 d) on uptake of Sr into the scales of juvenile rainbow trout (*Oncorhynchus mykiss*). Doubling and tripling the Sr concentration in the ambient water produced corresponding twofold and threefold increases in final scale (Sr). A twofold increase in exposure time produced nearly the same effect as a twofold increase in concentration. Widespread adoption of this technique will require establishment of optimal concentration and exposure times, validation of methods for compensating for Sr incorporated during seawater residence, and development of hatchery-specific chemical markers using combinations of several different elements.

Le strontium (Sr), qui est chimiquement semblable au calcium auquel il peut se substituer dans les tissus osseux des poissons, s'avère prometteur pour le marquage chimique, peu coûteux et efficace, des salmonidés juvéniles. Les auteurs ont étudié les effets de trois concentrations de Sr dans l'eau (1,8, 3,6 et 5,4 mg/L) et de deux périodes d'exposition (30 et 60 d) sur l'absorption de cet élément dans les écailles de truites arc-en-ciel juvéniles (*Oncorhynchus mykiss*). Le fait de doubler ou de tripler la concentration de Sr dans l'eau s'est traduit par des concentrations de Sr dans les écailles qui étaient de deux ou trois fois supérieures. L'augmentation par un facteur de deux de la durée d'exposition a presque donné les mêmes résultats que l'accroissement par deux de la concentration. L'utilisation généralisée de cette technique supposerait de déterminer les concentrations et les durées d'exposition optimales, de valider des méthodes pour tenir compte de l'absorption de Sr pendant la vie en mer et de mettre au point des marqueurs spécifiques aux piscicultures par l'utilisation de combinaisons d'éléments.

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Traditional techniques for marking fish, including fin clips, freeze brands, and coded wire tags, require handling of individual fish and are often difficult to administer to small fish or fry. Chemical marking, which involves incorporation of biologically rare elements or compounds into body tissues, could potentially provide an efficient means of marking large numbers of small fish. An ideal chemical mark would be relatively inexpensive, easily administered, harmless to fish, and long lasting.

Strontium (Sr), which is chemically similar to calcium and can substitute for this element in bony tissues such as vertebrae and scales, appears to meet many of these criteria. Dietary supplements of Sr have been used to mark goldfish (Ophel and Judd 1968) and salmon (Behrens Yamada et al. 1979; Behrens Yamada and Mulligan 1982). More recently, Behrens Yamada and Mulligan (1987) found that juvenile salmon incorporated Sr which had been added directly to ambient water and that the Sr tag induced in this manner was detectable after nearly 6 mo of growth.

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Widespread application of marking with Sr requires determination of appropriate concentrations and exposure times for use with this technique. This study examines the effects of three different Sr concentrations (1.8, 3.6, and 5.4 mg/L) and two different exposure times (30 and 60 d) on uptake of Sr into the scales of juvenile rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Juvenile rainbow trout with wet body weights of 0.7–1.3 g were obtained from West Creek Trout Farm (Aldergrove, B.C., Canada) in January 1990. Five fish were added at random to each of twelve 38-L aquaria, which were subsequently assigned in pairs to either a control group or one of 5 Sr treatment groups. Treatment groups consisted of 30-d incubations in low (1.8 mg/L), medium (3.6 mg/L), or high (5.4 mg/L) Sr concentrations and 60-d incubations in low (1.8 mg/L) and medium (3.6 mg/L) Sr concentrations. Treatment water was prepared by dissolving strontium chloride in tap water dechlorinated with activated carbon filters; treated water (as well as untreated water

for the control aquaria) was aerated and left standing a minimum of 24 h before being used.

During Sr treatment periods, 19 L of water from each tank was drained twice weekly and replaced with aged water of the same Sr concentration; inside box filters containing filter wool were used to maintain suitable water quality. After treatment periods ended, tanks were converted to a flow-through system delivering a continuous supply of dechlorinated tap water to each tank. Treatment periods began on 5 February 1990 and ended 7 March 1990 for the 30-d treatments and 6 April for the 60-d treatments. Water temperatures were maintained at 10–12°C throughout the experiment, and each aquarium received 0.15 g of semimoiest starter diet (Moore Clarke, La Connor, Washington) twice daily. If mortality occurred in a tank over the course of the study, rations for that tank were reduced by 0.03 g per feeding for each observed mortality for the duration of the experiment.

All fish were removed from tanks on 30 April 1990, stunned, and weighed. A scalpel was used to remove as many scales as possible from each fish. Scrapings remained overnight in 12 × 75 mm glass test tubes half filled with distilled water. Test tubes were then vortexed, centrifuged 30 s at approximately 100 × g, and decanted. This rinsing procedure (distilled water, vortex, spin, decant) was repeated 10 times for each sample. Test tubes were then carefully inverted and allowed to air-dry at room temperature for 4 d.

The dried scale samples, ranging in weight from 0.0003 to 0.0096 g, were placed in 15-mL polypropylene centrifuge tubes to which 400-μL aliquots of ultrapure concentrated nitric acid were added. The tubes were loosely capped and incubated in a water bath at 100°C for 2 h, allowed to cool, and then filled with distilled water to make up a final volume of 10 mL. The samples were presented to an inductively coupled plasma mass spectrometer (Plasmaquad model I; VG, England) for Sr analysis, using calcium (Ca) as a natural internal standard with a nominal concentration of 10%. Using an internal Ca standard in this way controls for differences in initial weight among samples.

Differences in initial body weight, final body weight, and final scale Sr concentrations were analyzed using SAS PROC GLM (SAS Institute Inc. 1985) for an analysis of variance. Sources of variation in the model were treatment and replicated (nested within treatments), and means were compared using the REGWQ multiple range test option. Significance was accepted at the $p < 0.05$ level. Since only two different exposure times were used in this study (30 and 60 d), no attempt was made to model the relationship between final scale (Sr) and exposure time.

Results and Discussion

Average body weights at the initiation and termination of the study were 0.89 and 2.78 g, respectively, and mortality was distributed fairly evenly across treatments (Table 1). Initial and final body weights did not differ significantly among treatments.

Final scale (Sr) in all treatment groups was significantly higher than in the control (Fig. 1). The low 30-d treatment resulted in significantly lower final scale (Sr) than the medium and high 30-d treatments, while the latter two did not differ significantly from each other. The difference in final scale (Sr) between the two 60-d treatments was significant, and the 60-d exposures resulted in significantly higher scale (Sr) than 30-d exposures with the same treatment (Sr).

TABLE 1. Initial average body weights ($\pm$ SE), final average body weights ($\pm$ SE), number of fish surviving, and average scale Sr concentration ($\pm$ SE) per aquarium. Each aquarium initially contained five fish. Treatment groups consisted of 30-d exposures in low (L) (1.8 mg/L), medium (M) (3.6 mg/L), or high (H) (5.4 mg/L) Sr concentrations and 60-d exposures in low (L) (1.8 mg/L) and medium (M) (3.6 mg/L) Sr concentrations.

Tank No.	Treatment	Initial weight (g)	Final weight (g)	No. of fish surviving
1	Control	0.84 $\pm$ 0.05	2.60 $\pm$ 0.46	4
2	Control	0.83 $\pm$ 0.09	2.55 $\pm$ 0.49	4
3	L, 30 d	0.83 $\pm$ 0.05	2.68 $\pm$ 0.63	4
4	L, 30 d	0.90 $\pm$ 0.06	2.78 $\pm$ 0.58	4
5	M, 30 d	0.84 $\pm$ 0.07	2.70 $\pm$ 0.64	3
6	M, 30 d	0.86 $\pm$ 0.05	3.07 $\pm$ 0.53	4
7	H, 30 d	0.87 $\pm$ 0.07	2.62 $\pm$ 0.46	5
8	H, 30 d	0.87 $\pm$ 0.04	2.88 $\pm$ 0.54	4
9	L, 60 d	0.96 $\pm$ 0.09	3.90 $\pm$ 1.31	2
10	L, 60 d	0.95 $\pm$ 0.08	2.70 $\pm$ 0.79	4
11	M, 60 d	0.96 $\pm$ 0.04	2.53 $\pm$ 0.73	4
12	M, 60 d	0.95 $\pm$ 0.06	3.08 $\pm$ 0.52	4

A twofold increase in exposure time produced nearly the same final scale (Sr) as a twofold increase in concentration (compare low and medium 60-d treatments with medium and high 30-d treatments, respectively).

The results of this study, along with earlier work by Behrens Yamada and Mulligan (1987), demonstrate the feasibility of marking juvenile salmonids with dissolved Sr. The strong correlation between scale (Sr) and both treatment (Sr) and exposure time suggests that the substitution of Sr for Ca in the scales occurs in a regular and predictable fashion. Establishing an optimal dose and exposure time for this technique will require a balancing of several factors, including the desirability of producing a well-defined mark versus the relative cost of the marker element. Since there is evidence that high levels of dietary Sr may induce cataracts in some salmonids (Richardson et al. 1986), use of relatively low dissolved Sr concentrations would be prudent.

It is possible that either lower concentrations of Sr or shorter exposure times may be used than our lowest values (treatment [Sr] of 1.8 mg/L and exposure time of 30 d) with a satisfactory signal-to-noise ratio. Possibly, a 20-d exposure with a Sr concentration of approximately 2.0 mg/L would be a reasonable compromise between strength of the induced Sr mark versus minimizing any possible deleterious effects of the dissolved Sr. Given the fact that our medium concentration treatment (3.6 mg/L) with 60-d exposure and our high concentration treatment (5.4 mg/L) with 30-d exposure produced no observable negative effects on the fish, the suggested treatment concentration of 2.0 mg/L over 20 d would likely be safe and effective.

In addition to Sr added purposely to treatment tanks, fish in our experiment were exposed to two other sources of Sr: trace Sr present in the laboratory water supply and Sr present in the prepared trout diet. Fairly high levels of Sr (100–500 μg/g) may be present in prepared fish diets (T. J. Mulligan, pers. comm.), and salmonids can incorporate dietary Sr into their scales and vertebrae (Behrens Yamada et al. 1979). The low final scale (Sr) of our control fish indicates, however, that Sr from dietary and trace water sources would be expected to make a minor contribution to final scale (Sr) compared with our suggested treatment of 2.0 mg/L of dissolved Sr over a 20-d exposure.

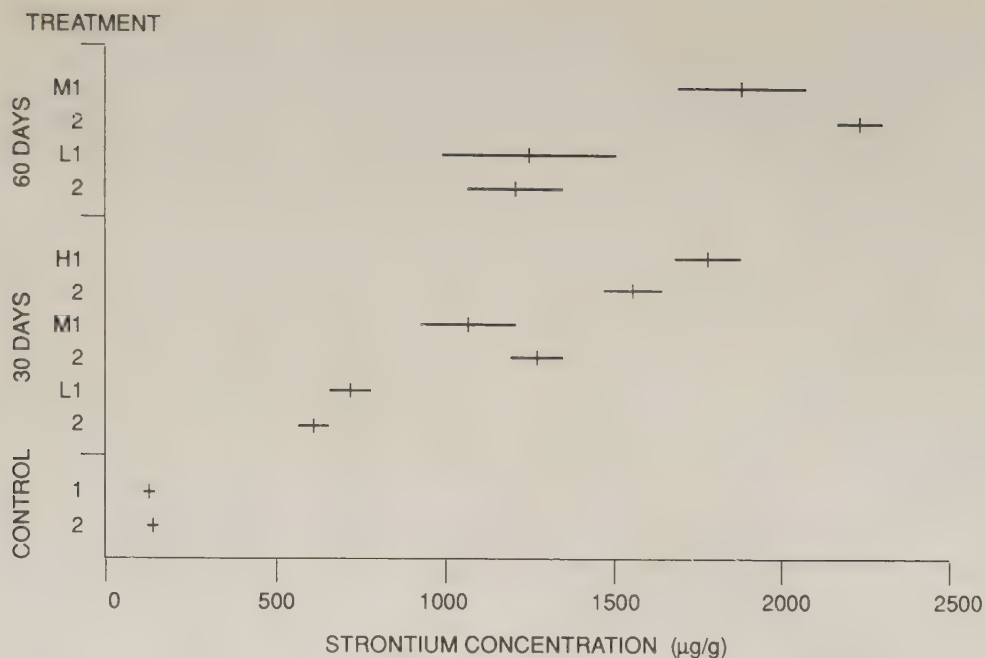


FIG. 1. Sr concentration in scale samples of juvenile rainbow trout. Treatment groups consisted of 30-d exposures in low (L) (1.8 mg/L), medium (M) (3.6 mg/L), or high (H) (5.4 mg/L) Sr concentrations and 60-d exposures in low (L) and medium (M) Sr concentrations. Values presented for each treatment and the control are means (vertical line) of two replicates. Horizontal lines indicate standard errors of the means.

As discussed by Behrens Yamada and Mulligan (1982, 1987), use of Sr marking in anadromous salmonids can be complicated by the fact that induced Sr marks will tend to be masked by Sr absorbed from seawater. These authors compensated for this potential masking effect by using a leather punch to isolate the core region of returning adults' scales before analyzing samples for Sr content. This indicates that possible dilution of the induced Sr mark during seawater residence does not present a serious technical obstacle, and we are currently exploring means of analyzing the core region of scales with even more precision than earlier methods would allow.

It is anticipated that marking hatchery salmon with dissolved Sr, and perhaps other biologically rare elements such as samarium and rubidium, will provide an efficient and cost-effective means for tagging large numbers of young fish. This method should be superior to the use of dietary Sr to label fish, since special feeds would not have to be prepared. Screening of adult returns would require only the collecting of scale samples, a procedure which is easily performed and noninvasive. We are currently establishing protocols for the laboratory determination of scale elemental composition which will minimize sample preparation requirements as well as final cost per sample. With full development of this technique, hatchery operators should not only be able to easily distinguish wild from hatchery

fish, but unique hatchery marks based on different combinations of elements should also allow operators to assign adult returns to specific hatcheries with precision.

Acknowledgements

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Seasonal Changes in Habitat Use by Juvenile Coho Salmon (*Oncorhynchus kisutch*) in Oregon Coastal Streams

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Habitat use by juvenile coho salmon (*Oncorhynchus kisutch*) during spring, summer, and winter was examined in Oregon coastal streams. Coho salmon fry were most abundant in backwater pools during spring. During summer, juvenile coho salmon were more abundant in pools of all types than they were in glides or riffles. During winter, juvenile coho salmon were most abundant in alcoves and beaver ponds. Because of the apparent strong preference for alcove and beaver pond habitat during winter and the rarity of that habitat in coastal streams, we concluded that if spawning escapement is adequate, the production of wild coho salmon smolts in most coho salmon spawning streams on the Oregon Coast is probably limited by the availability of adequate winter habitat.

L'habitat utilisé par les juvéniles du saumon coho (*Oncorhynchus kisutch*) pendant le printemps, l'été et l'hiver a été étudié dans des cours d'eau côtiers de l'Orégon. Au printemps, les alevins étaient les plus abondants dans les fosses d'eau calme. Pendant l'été, les juvéniles étaient plus abondants dans tous les types de fosses plutôt que dans les descentes et sur les hauts fonds. Pendant l'hiver, les juvéniles étaient les plus abondants dans les enfoncements latéraux et les étangs de castors. Étant donné la forte préférence apparente pour les enfoncements latéraux et les étangs de castors pendant l'hiver et la rareté de ces habitats en cours d'eau côtiers, les auteurs concluent que si la remontée de géniteurs est suffisante, la production de smolts de saumon coho sauvage dans la plus grande partie des cours d'eau à coho de la côte de l'Orégon est probablement limitée par la disponibilité d'habitats hivernaux adéquats.

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During strategic resource planning, biologists often estimate the smolt production potential of a stream or basin for anadromous salmonids without the benefit of measures of smolt outmigration (e.g. Columbia Basin Fish and Wildlife Authority 1990; USDA Forest Service 1990; land management plans currently being developed by the U.S. Bureau of Land Management for western Oregon). The most common method being used to assess the potential of a stream to produce smolts is to apply a density (smolts per square metre) to estimates of the summer surface area of the stream (Columbia Basin Fish and Wildlife Authority 1989, 1990). This method relies on the assumption that all habitat types (i.e. riffles, pools, glides) have the same potential or, when pool area is estimated separately, that all types of pools have the same potential. The primary weakness of this method is that it assumes that smolt production is limited by summer surface area, thus totally ignoring the quantity and quality of habitat during other times of the year.

Recent advances in stream habitat classification (Bisson et al. 1982) and habitat inventory methods (Hankin and Reeves 1988) have made it possible to easily estimate the quantity of different types of habitat in streams during different seasons of the year. The habitat classification also provides a framework for evaluating habitat quality. The purpose of this study was to use that framework to determine the types of stream habitat used by juvenile coho salmon (*Oncorhynchus kisutch*) inhabiting Oregon coastal streams during spring, summer, and winter and to quantify the density of juvenile coho salmon associated with different types of habitat.

A number of investigators have examined the habitat preferences of coho salmon in recent years in Alaska (Heifetz et al.

1986; Murphy et al. 1986, 1989), British Columbia (Bustard and Narver 1975a; Tschaplinski and Hartman 1983; Swales et al. 1986, 1988; Hartman and Brown 1987; Swales and Levings 1989), and Washington (Bisson et al. 1982, 1988; Peterson 1982a, 1982b). However, few of these papers present data on coho salmon density in different stream habitats, and little has been published about the seasonal habitat preferences of juvenile coho salmon in the southern portion of their range.

Methods

Study Design

Our approach was to sample different types of natural habitat over a broad range of streams to determine (1) the average density of juvenile coho salmon associated with each type of habitat and (2) the extent to which that density varied seasonally. We sampled specific habitat types and fish populations in streams located in Oregon coastal river basins from the Nehalem River south to the Coquille River (Fig. 1), an area that included most of the range of coho salmon on the Oregon coast (Oregon Department of Fish and Wildlife 1982).

Because we intended that our density estimates reflect the average potential of different types of habitat to support juvenile coho salmon in Oregon coastal streams, it was necessary for the streams that we sampled to be adequately seeded. The inclusion of underseeded streams, and therefore low densities of coho salmon, would result in an underestimate of the habitat potential and also could mask any differences in the relative use of different types of habitat. Unfortunately, many Oregon coastal streams have had poor spawning escapements since the

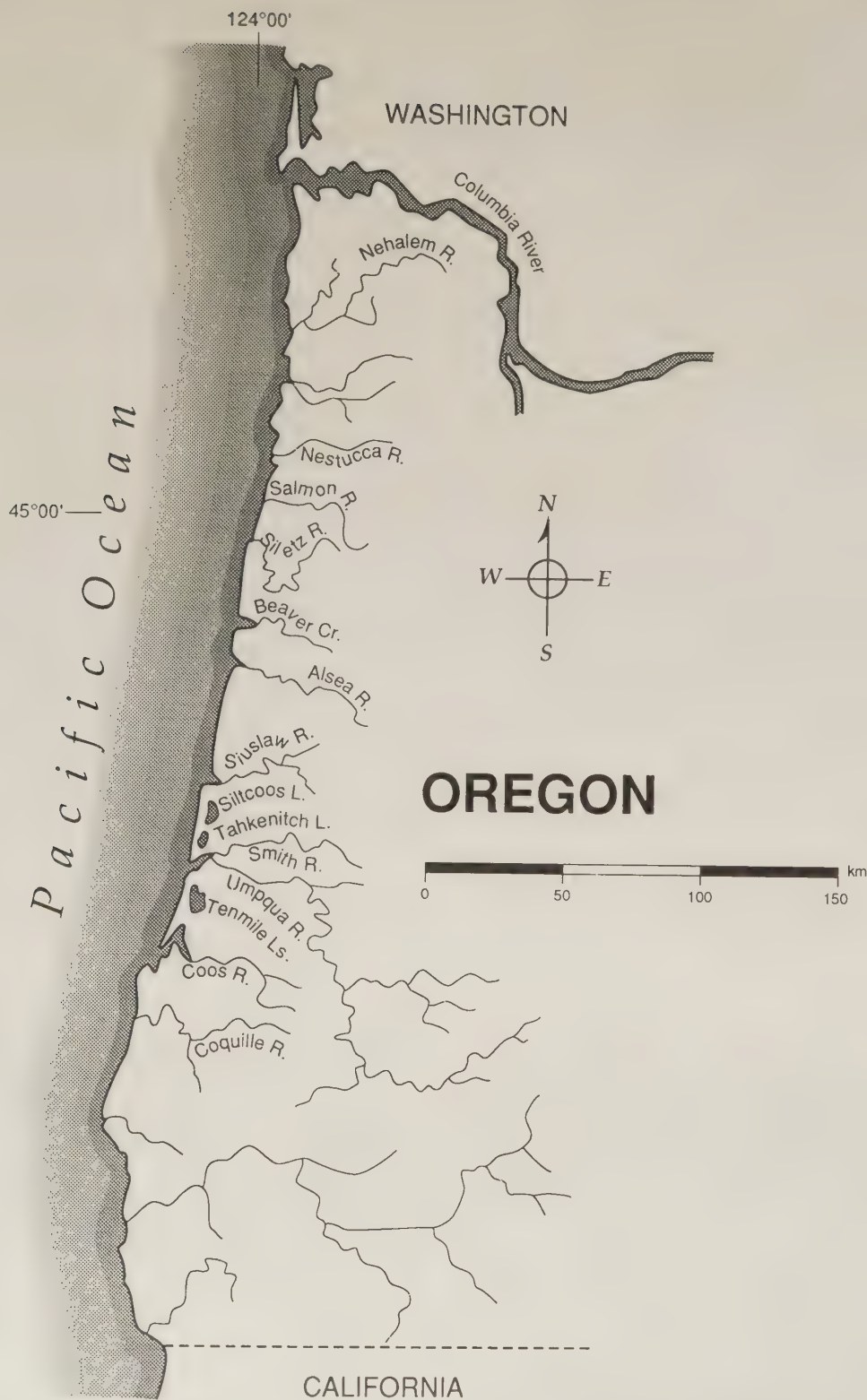


FIG. 1. Coastal Oregon basins where juvenile coho salmon populations and habitat were sampled.

mid-1970s (Oregon Department of Fish and Wildlife 1982; Jacobs 1989), increasing the likelihood of underseeded juvenile rearing habitat.

To reduce the possibility that we would sample underseeded streams, we sampled streams during the spring that had spawning populations of at least 25 spawners/km the previous fall (Jacobs 1989), a level of spawners that should be adequate to seed juvenile rearing habitat (Beidler et al. 1980). However,

because information on spawner abundance is limited to a few streams, and we sampled many more streams during summer than during spring, the seeding level of many of the streams that we sampled during summer was unknown. Therefore, for our analysis of summer habitat use, we included only streams that had an average density in pools of at least 1.0 fish/m². Earlier work with stocking levels of juvenile coho salmon (Nickelson et al. 1986) suggested that this density would result

TABLE 1. Stream habitat types as modified from Bisson et al. (1982).

Habitat type	Characteristics
Pools	
Dammed pool	A pool impounded upstream from a complete or nearly complete channel blockage (including beaver ponds)
Backwater pool	An eddy or slack water along the channel margin separated from the main current by a gravel bar or small channel obstruction
Alcove	A slack water along the channel margin separated from the main current by streambanks or large channel obstructions such that it remains quiet even at high flows
Scour pool	A scoured basin either (1) near the channel margin caused by flow being directed to one side of the stream by a partial channel obstruction or (2) near the center of the channel usually caused by a channel constriction or high-gradient rapid
Plunge pool	A basin scoured by a vertical drop over a channel obstruction
Trench pool	A long, usually deep slot in a stable substrate (often bedrock)
Glide	A moderately shallow reach with an even flow and no pronounced turbulence
Riffles	
Riffle	A shallow reach of gradient $\leq 4\%$ with moderate current velocity and moderate turbulence
Rapid	A shallow reach of gradient $> 4\%$ with high current velocity and considerable turbulence
Cascade	A series of small steps of alternating small waterfalls and small pools

in full occupation of rearing space in all pools. To insure an adequate number of juvenile coho salmon to seed winter habitat, we resampled streams during the winter that had an average density during summer of at least 0.7 fish/m². We found average densities during winter to be similar between streams regardless of whether average summer densities were 0.7–1.0 or ≥ 1.0 fish/m², indicating that even the lower density streams appeared to be fully seeded in winter.

Classification of Habitat Types

We use the method described by Bisson et al. (1982) to classify habitat components. This method sorts stream habitat into three basic categories: pools, riffles, and glides. It further divides pools and riffles into easily identifiable types based on their hydraulic characteristics (Table 1).

We made two modifications to the Bisson classification scheme. First, in addition to the lateral scour pool they described, we identified a midchannel scour pool. It is characterized by a downward scour that results in a bowl shape as opposed to a lateral scour pool that tends to be deep on one side. We combined midchannel and lateral scour pools into a single category: scour pool (Table 1). Second, we classified secondary channels, which were rare, by their appropriate habitat type and treated them the same as main-channel habitats because they were just smaller versions of the main-channel habitats with similar hydraulic characteristics.

We also classified backwater pools during winter into two types: (1) those with low current velocity, even at high flows, which we termed “alcoves” after Helm (1985), and (2) those with moderate to high current velocity during high flow, for which we retained the term “backwater pool.” Alcoves are formed by impounded water backing up into quiet water areas along the channel margin and are protected by the streambank or by large channel obstructions whereas backwater pools are formed by eddies behind small channel obstructions and are often separated from the main current by gravel bars only. Alcoves were treated separately from backwaters only during winter because the distinction is only important at high flow, they tend to be rare, and they are often dry at summer flow levels.

Study Area

A typical study stream had low gradient ($< 3\%$) and was dominated by scour pools and riffles. Stream order (Strahler 1957) ranged from second to sixth. The riparian zone generally consisted of an overstory of red alder (*Alnus rubra*), bigleaf maple (*Acer macrophyllum*), western redcedar (*Thuja plicata*), Douglas-fir (*Pseudotsuga menziesii*), and western hemlock (*Tsuga heterophylla*) and an understory of salmonberry (*Rubus spectabilis*), salal (*Gaultheria shallon*), vine maple (*A. circinatum*), and sword fern (*Polystichum munitum*). Typical summer and winter water temperatures ranged between 11 and 17°C and between 4 and 9°C, respectively. In addition to coho salmon, steelhead (*O. mykiss*), coastal cutthroat trout (*O. clarki*), and sculpins (*Cottus* spp.) were usually present.

Sampling Techniques

Sampling took place following fry emergence during spring (March–May), during the summer low flow period (August–mid-October), or during winter following a bankfull flow event (December–mid-February). We sampled 15 streams and 150 habitat units during spring in 1986, 1987, and 1989, 52 streams and 519 habitat units during summer in the years 1985–90, and 31 streams and 289 habitat units during winter in the years 1985–89.

We estimated the population of juvenile coho salmon in each habitat unit using either a mark–recapture census (Chapman 1951) or a two- or three-pass removal estimate (Seber and LeCren 1967). The upper and lower ends of the unit were blocked with seines, and fish were collected using electrofishing equipment and seines. We estimated the surface area of each unit by measuring the length and multiplying by the average of several width measurements. During winter, we also measured the maximum depth of each pool.

Analysis

We have found that mark–recapture population estimates are more accurate than removal population estimates, accounting for 30% more of a coho salmon population than do the removal estimates (Rodgers et al. 1992). Therefore, to standardize our population estimates, we multiplied all removal population estimates by 1.3 to make them consistent with the mark–recapture population estimates. This affected about 30% of our data. All population estimates were converted to density (fish per square metre).

We calculated standard descriptive statistics for the coho salmon density for each habitat type except cascades for each of the three seasons. Cascades were excluded because of the

extremely small number sampled (two in summer, three in winter). Cascades are uncommon in the low-gradient streams coho salmon typically inhabit.

We used a natural logarithm transformation on the various data when necessary to correct for nonnormal distributions or heterogeneous variances (Snedecor and Cochran 1967; Sokal and Rohlf 1969). This transformation normalized the densities for all seasons and equalized the variances among habitat types for spring (Bartlett's test, $P > 0.05$). However, the transformation would not equalize the variances among habitat types for summer or winter (Bartlett's test, $P \leq 0.01$).

We used analysis of variance (ANOVA) to test the hypothesis of no difference in mean density of juvenile coho salmon among habitat types within each season. When ANOVA F tests were significant, we followed them with a stepwise paired comparison. We used Ryan's Q test (Einot and Gabriel 1975), as recommended by Day and Quinn (1989), to compare pairs of means of the transformed habitat-specific densities of coho salmon when variances were homogeneous. We used Games-Howell (GH) test (Games and Howell 1976; Day and Quinn 1989) when variances were heterogeneous. This test was used because the pooled estimate of variance from the ANOVA used in Ryan's Q test should not be used to calculate the standard error of comparisons when the treatment variances are heterogeneous (Day and Quinn 1989). The GH test resolved this problem by calculating an appropriate standard error for each comparison.

To examine the influence of maximum depth on density of juvenile coho salmon during the winter, we used correlation analysis (Sokal and Rohlf 1969).

Results and Discussion

Seasonal Habitat Use

We found highly significant differences in mean density among habitat types in all seasons (ANOVAs on transformed data, $P \leq 0.001$). Juvenile coho salmon were most abundant in pool habitat throughout the year (Fig. 2). However, the type of pool with the highest density varied by season.

During spring, the mean density of coho salmon fry in backwater pools was more than twice that of any other habitat type (Ryan's Q test, $P \leq 0.05$), and mean density in dammed pools, glides, and scour pools was greater than that in rapids (Ryan's Q test, $P \leq 0.05$, Fig. 2). Lister and Genoe (1970) also found that coho salmon fry prefer backwater areas, and Peterson and Reid (1984) found coho salmon fry moving from the mainstem Clearwater River, Washington, into off-channel, slack-water areas (i.e. wall-base channels and ponds). Although we found fry to be most abundant in backwater pools, they were common in all habitat types, even riffles and rapids, where they used the stream margins (Mundie 1969; Lister and Genoe 1970).

During summer, there was no difference in mean density of juvenile coho salmon among pool types (GH test, $P > 0.05$, Fig. 2), although backwater pools had the lowest mean density of all pool types. We also found that riffle habitat supported the lowest density of juvenile coho salmon during summer and that glides were intermediate in density between riffles and pools (GH test, $P \leq 0.05$). These results are similar to those found by Ruggles (1966) in an artificial stream channel. In Washington streams, Bisson et al. (1988) found that abundance of juvenile coho salmon was greater in pools than in riffles and rapids. However, they found that abundance of juvenile coho salmon in glides was similar to that in riffles and rapids, although their sample size for glides was small ($n = 4$).

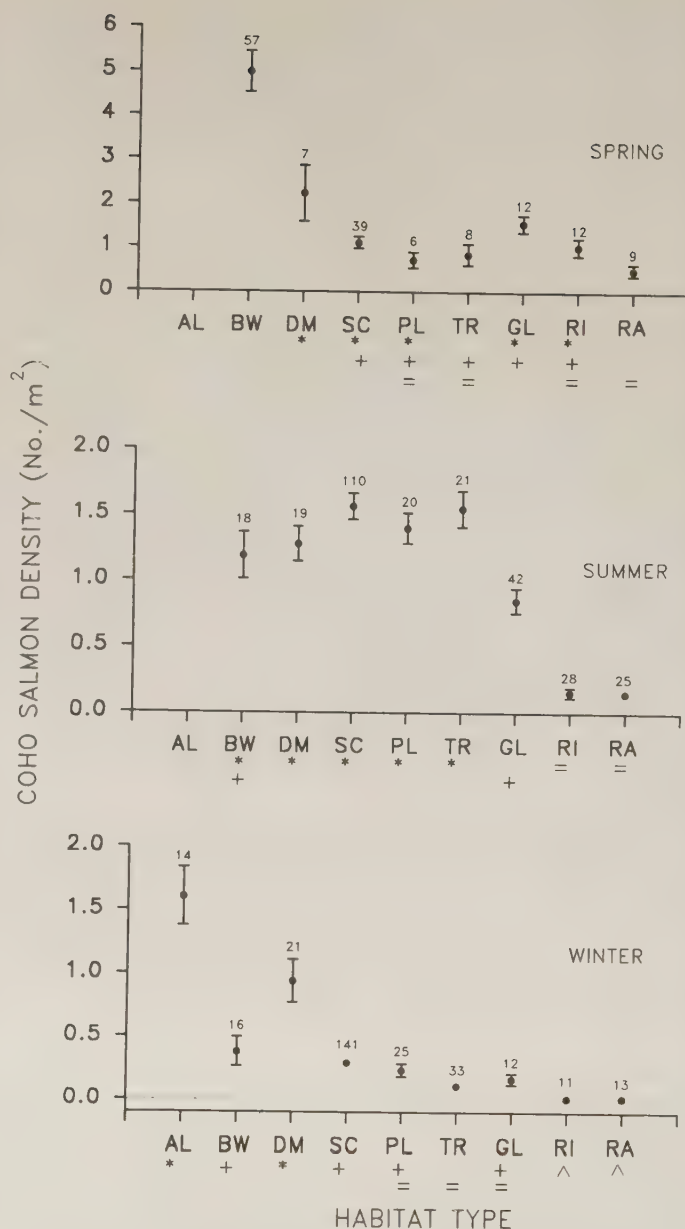


FIG. 2. Mean and standard error for density of juvenile coho salmon by habitat type during spring, summer, and winter. Some standard errors are smaller than the points. Habitat types are arranged from slowest to fastest water velocities after Bisson et al. (1988). AL = alcove; BW = backwater pool; DM = dammed pool; SC = scour pool; PL = plunge pool; TR = trench pool; GL = glide; RI = riffle; RA = rapid. Like symbols below these abbreviations indicate habitat types that had mean densities not significantly different from one another ($P > 0.05$). Sample size is shown above each datum.

During winter the mean density of juvenile coho salmon in alcoves and in dammed pools, habitats with low current velocity and little turbulence during freshets, was greater than that in all other habitat types (GH test, $P \leq 0.05$, Fig. 2). As in summer, mean salmon density in riffles and in rapids was significantly lower than that in all other habitat types (GH test, $P < 0.05$).

Of the 21 dammed pools sampled during winter, 12 were beaver ponds. The distinction between beaver ponds and other dammed pools during winter is an important one. Compared with other dammed pools, beaver ponds supported more fish (mean = 456/pond versus 96/pool; ANOVA, $P \leq 0.01$) at a

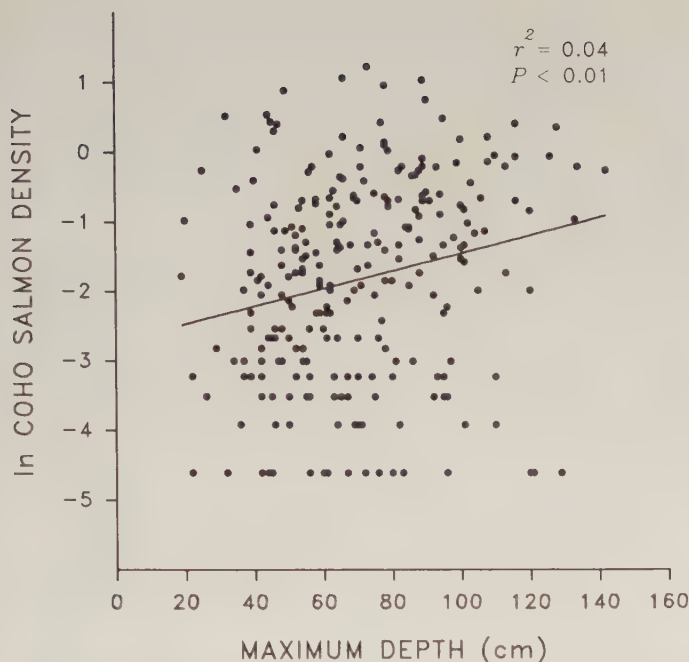


FIG. 3. Relationship between density of juvenile coho salmon during winter and maximum pool depth.

TABLE 2. Correlation coefficients (r), sample size (n), and probability (P) for relationships between density of juvenile coho salmon during winter (after natural logarithm transformation) and maximum depth of pools.

Pool type	Average maximum depth (cm)	r	n	P
All pools	70	0.21	250	<0.01
Alcove	63	0.12	14	0.69
Backwater	56	-0.43	16	0.10
Dammed	92	0.45	21	0.04
Scour	66	0.41	141	<0.01
Plunge	79	-0.28	25	0.18
Trench	72	0.31	33	0.08

higher density (1.28 versus 0.49 fish/m²; ANOVA, $P \leq 0.01$). The beaver ponds were also larger (450 versus 150 m²; ANOVA, $P \leq 0.01$) and deeper (103 versus 77 cm; ANOVA, $P \leq 0.01$) than the other dammed pools. Dammed pools tend to be an area of deposition, and thus fill in through time. Beavers maintain depth in their ponds by continually increasing the height of their dams and by creating channels of deeper water (Naiman et al. 1988).

Maximum pool depth during winter was significantly correlated ($P \leq 0.01$) with density of juvenile coho salmon when all pool types were analyzed collectively. However, this relationship was highly variable, and maximum depth explained only 4% of the variation in density of juvenile coho salmon (Fig. 3). Except for alcoves, the individual correlation coefficients were higher when the density – maximum depth relationship was analyzed by pool type (Table 2). Maximum pool depth was positively correlated with density of juvenile coho salmon in scour pools ($P \leq 0.01$), dammed pools ($P = 0.04$), and trench pools ($P = 0.08$). In these pool types, depth may be a factor that reduces current velocity and turbulence. Maximum pool depth was negatively correlated with density of juvenile coho salmon in plunge pools and

backwater pools. During high flow, these pools undergo a downward scouring action. Greater depth may be an index of greater velocity and turbulence in backwater and plunge pools during freshets. Depth is not an important factor influencing the density of juvenile coho salmon found in alcoves. Alcoves are located out of the main channel and thus have low current velocity regardless of depth.

Winter habitat of coho salmon in Oregon coastal streams is similar to that reported by investigators elsewhere. Bustard and Narver (1975a, 1975b) found that during winter, juvenile coho salmon prefer natural or artificial “sidepools,” similar to the habitat we classified as alcoves. They also found that beaver ponds were important overwintering habitat. Everest et al. (1986) sampled beaver ponds on an Oregon Cascade Mountain stream and found densities similar to those we report. Bustard and Narver (1975a), Tschaplinski and Hartman (1983), and Hartman and Brown (1987) observed movement of juvenile coho salmon from Carnation Creek, British Columbia, into slow off-channel habitats during September–December. The use of off-channel ponds and lakes during winter has been observed in Washington (Peterson 1982b; Peterson and Reid 1984) and British Columbia (Swales et al. 1986, 1988; Swales and Levings 1989).

We observed definite seasonal shifts in habitat use by juvenile coho salmon. Between spring and summer the shift was primarily from the riffles, glides, and backwater pools to other types of pools. This movement was likely the result of reduced velocities in main-channel pools as a result of lower streamflows during summer and a shift to deeper water as the fish grew (Lister and Genoe 1970). The shift in habitat use was most dramatic from summer to winter, probably a response to increased streamflows and decreased water temperature (Bustard and Narver 1975a; Tschaplinski and Hartman 1983). Many scour, trench, and plunge pools that supported large numbers of coho salmon during summer supported few, if any, during winter. Alcoves and beaver ponds composed only 9% of the total number of habitat units and 31% of the area sampled during winter, but accounted for 66% of the coho salmon sampled.

The types of habitat used by juvenile coho salmon during winter were once quite common in Oregon coastal streams (Sedell and Luchessa 1982). During the nineteenth century, coastal streams and rivers were choked with wood and beaver ponds that provided varied and complex habitat for anadromous salmonids, including numerous marshes, sidechannels, and sloughs. Most of this diversity has been lost as a result of clearing, splash-damming, diking, and channelizing streams associated with timber harvest, log transport, and agriculture (Sedell and Luchessa 1982). Today, alcoves and beaver ponds are rare in Oregon coastal streams during winter (Table 3), leading us to believe that given adequate seeding, production of coho salmon smolts in many Oregon coastal streams is probably limited by the availability of winter habitat.

Estimating Production Potentials and Limiting Factors for Coho Salmon

An understanding of the habitat associations of anadromous salmonids throughout the year is necessary to identify factors limiting smolt production and to assess the capacity of a stream or basin to produce smolts. Identification of limiting factors is a critical element in the planning of stream habitat rehabilitation projects (Hall and Baker 1982). This element is often missing because of the lack of analytical methods to identify limiting factors. Lack of information on the density of salmonids in

TABLE 3. Occurrence of winter habitat classified as prime (alcoves and beaver ponds), fair (dammed, backwater, and scour pools), or poor (plunge and trench pools, glides, riffles, and rapids) for production of coho salmon in 14 Oregon Coast Range streams as determined from stream inventories conducted at winter base-flow.

Stream	Length (km)	Percent of winter habitat by area		
		Prime	Fair	Poor
Moon Cr.	4.4	0.1	21.4	78.5
East Cr.	5.0	0.0	24.6	75.4
E. F. Lobster Cr.	3.4	2.3	25.7	72.0
S. F. Lobster Cr.	3.8	0.1	23.9	76.0
Benson Cr.	16.4	12.4	11.3	76.3
Schooner Cr.	4.7	0.0	24.8	75.2
Carcus Cr.	5.1	0.3	19.0	80.7
Hortil Cr.	0.6	0.0	36.3	63.7
Klickitat Cr.	0.3	0.0	30.1	69.9
Staveboldt Cr.	1.3	0.0	23.0	77.0
Speelyai Cr.	0.9	0.0	38.6	61.4
Little Cr.	4.3	0.5	23.4	76.1
Raymond Cr.	3.5	0.1	15.8	84.1
Walford-Johnson Cr.	0.9	11.9	8.2	79.9

TABLE 4. Potential populations of coho salmon supported seasonally as estimated from stream inventories and habitat-specific mean densities (see text).

Stream	Estimated potential population			
	Eggs	Spring	Summer	Winter
Moon Cr.	448 400	22 500	13 700	2 400
East Cr.	1 961 600	39 900	15 900	4 400
E. F. Lobster Cr.	1 330 000	28 100	12 900	3 900
S. F. Lobster Cr.	1 335 000	32 600	15 900	3 800
Benson Cr.	1 615 600	139 900	57 000	30 200
Schooner Cr.	801 600	28 600	13 800	3 200
Carcus Cr.	414 200	40 600	9 000	3 100
Hortil Cr.	91 600	2 200	1 100	300
Klickitat Cr.	5 000	2 500	1 500	100
Staveboldt Cr.	120 000	5 200	5 100	400
Speelyai Cr.	5 000	6 200	2 100	800
Little Cr.	570 000	23 500	8 500	2 400
Raymond Cr.	82 600	13 200	4 700	800
Walford-Johnson Cr.	51 600	13 100	4 900	2 100

various types of habitat during different times of the year has impeded the development of such methods.

Results from this study can be used to interpret stream habitat inventories in terms of the capacity of the habitat to produce coho salmon smolts and to develop methods to identify factors that limit production of coho salmon smolts. The Bisson et al. (1982) habitat classification system is useful for accomplishing these products because it is detailed enough to differentiate among preferences of coho salmon, yet can easily be used by a field crew conducting basinwide surveys.

Because of the substantial seasonal changes in habitat use by juvenile coho salmon, it is difficult, if not impossible, to predict the smolt production potential of a stream based only on an inventory of summer habitat, as is the common practice today, or to reliably determine the habitat limiting production. Provided that water quality is adequate, habitat potential during summer is largely dependent on the amount of pool habitat available. Habitat capacity during winter, however, is dependent on the amount of alcoves and dammed pools (especially

beaver ponds) available, much of which cannot be adequately estimated from a summer inventory because alcoves are generally dry at low summer flow and many beaver dams present in summer are washed out by the first fall freshet. We therefore recommend that stream habitat be inventoried during the summer low-flow period and again during a winter base-flow period. From this information, a stream's production potential and the habitats limiting production can more accurately be determined.

The capacity of a stream to support coho salmon during a particular season is the sum of the potential number of fish supported by each of the individual habitat units comprising the stream. This can be estimated by multiplying the average density of coho salmon that we observed for each habitat type by the area of that habitat as estimated from summer and winter stream habitat inventories. We recommend using the winter base-flow inventory to also estimate the potential of spawning habitat and of spring habitat. Table 4 provides an example of this approach applied to the same streams as shown in Table 3. In this case, we have estimated the egg potential by multiplying the estimated area of spawning gravel by 833 eggs/m² (Reeves et al. 1989).

We have used this basic approach of comparing the habitat needs of coho salmon for spawning, spring rearing, summer rearing, and winter rearing combined with survival rates between successive life stages to address the question of identification of limiting factors. A preliminary method, in the form of a key, has been developed to identify limiting factors (Reeves et al. 1989). We are currently testing and refining this method.

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Effectiveness of Selected Stream Improvement Techniques to Create Suitable Summer and Winter Rearing Habitat for Juvenile Coho Salmon (*Oncorhynchus kisutch*) in Oregon Coastal Streams

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We examined the use of constructed pools by juvenile coho salmon (*Oncorhynchus kisutch*) during summer and winter. Log, gabion, and rock structures placed across the full stream width provided good summer habitat but poor winter habitat for juvenile coho salmon. Rearing densities in constructed habitats during summer and winter were generally similar to those in natural habitats of the same type, except that constructed dammed pools supported lower densities during winter than natural dammed pools. The addition of brush bundles to pools created by full-stream-width structures increased the density of juvenile coho salmon in dammed pools during winter, but not in plunge pools. We concluded that the development of off-channel habitat has the greatest potential to increase production of wild coho salmon smolts in Oregon coastal streams.

Nous avons examiné l'utilisation de bassins artificiels par les juvéniles de saumons cohos (*Oncorhynchus kisutch*), pendant l'été et l'hiver. Des structures de bois rond, de clayonnages et de roches, placées à travers toute la largeur d'un cours d'eau, créent un bon habitat d'été, mais un habitat d'hiver médiocre pour les juvéniles de saumons cohos. Au cours de l'hiver et de l'été, les densités d'élevage dans les habitats artificiels étaient généralement semblables à celles qu'on trouve dans les habitats naturels du même type, sauf que les densités des bassins endigués artificiels étaient inférieures à celles des bassins endigués naturels pendant l'hiver. L'addition de fagots aux bassins dont les structures s'étaient sur toute la largeur du cours d'eau y augmentait la densité des juvéniles de saumons cohos au cours de l'hiver, mais non dans les marmites de géants. Nous avons conclu que l'élaboration d'habitats à l'écart du chemin principal du cours d'eau présente des meilleures possibilités d'augmenter la production de smolts de saumons cohos dans les cours d'eau côtiers de l'Oregon.

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The idea that the addition of structure to a stream channel (often in the form of logs or some type of woody debris) would result in increased fish production was first developed for trout in Wisconsin and Michigan (Hubbs et al. 1932; Tarzwell 1937; White and Brynildson 1967; Hunt 1969). Over the last 30 yr, the use and general acceptance of stream habitat "improvement" techniques by land and fisheries management agencies have spread north and west across the United States and Canada and are now an accepted management technique in the Pacific Northwest (Hall and Baker 1982; Reeves and Roelofs 1982).

Some habitat improvement projects have produced impressive increases in the abundance of desired fish species, particularly trout in the Midwest (Shetter et al. 1949; Hunt 1971, 1976; White 1975; Hall and Baker 1982). However, habitat improvement may not always produce the desired results (Calhoun 1966; Hall and Baker 1982; Reeves and Roelofs 1982; Olsen et al. 1984).

Habitat improvement projects appear to fail in two ways: (1) the desired change in the habitat is not created or (2) the desired change in habitat fails to produce the desired change in the fish population. The first type of failure is an engineering problem and may be associated with structures washing out during high flow or with the installation of the wrong structure. The second type of failure is a biological problem caused by failure of the project planners to recognize and improve the

habitat that was limiting the fish population (Hall and Baker 1982). For example, we have shown that juvenile coho salmon (*Oncorhynchus kisutch*) use different types of habitat during different times of the year (Nickelson et al. 1992) and have suggested that the availability of winter habitat limits coho salmon smolt production in many Oregon coastal streams. In such streams, habitat improvement that does not create good winter habitat will fail to increase production of coho salmon smolts.

To adequately plan and implement effective stream habitat improvement, managers must understand the habitat requirements of the species of interest throughout the year, the type of habitat that is in shortest supply in the particular stream of interest, and what technique to use to create the habitat that is needed. Unfortunately, in the few instances where habitat improvements have been evaluated in Pacific Northwest streams, most have relied on fish population data collected only during summer months (Olsen et al. 1984; House and Boehne 1985, 1986; Petrosky and Holubetz 1986; Shirvell 1990), and only occasionally have they considered the value of constructed habitat during winter (Peterson 1985; Everest et al. 1986; Cederholm et al. 1988). As a result, our knowledge of the true effectiveness of various stream habitat improvement techniques to increase populations of anadromous salmonids in the Pacific Northwest is limited.

Various types of habitat improvement techniques aimed at increasing rearing density of juvenile coho salmon have been tried in Oregon coastal streams. These techniques have included placing structures made of wood, boulders, or even concrete across or partially across the stream channel, excavating off-channel alcoves, and placing wood or boulders of various types and configurations into small coastal streams.

The purposes of this paper are to (1) examine the types of rearing habitat created by various habitat improvement techniques, (2) compare the relative effectiveness of the habitat created by these techniques to support juvenile coho salmon during summer and winter, and (3) compare the density of juvenile coho salmon in constructed habitats with that of juvenile coho salmon in natural habitats of the same type.

Methods

Evaluation of Constructed Habitat

Study design

Our approach was to sample different types of constructed habitat over a broad range of streams to determine (1) the average density of juvenile coho salmon associated with each type of habitat and (2) the extent to which that density varied seasonally.

Because we intended that our density estimates reflect the average potential of different types of constructed habitat to support juvenile coho salmon in Oregon coastal streams, it was necessary for the streams that we sampled to be adequately seeded. The inclusion of underseeded streams, and therefore low densities of coho salmon, would result in an underestimate of the potential of the constructed habitat and also could mask any differences in the relative use of different types of habitat. Unfortunately, many Oregon coastal streams have had poor spawning escapements since the mid-1970s (Oregon Department of Fish and Wildlife 1982; Jacobs 1989), increasing the likelihood of underseeded juvenile rearing habitat.

We chose streams to sample during summer based only on the presence of the constructed habitat, without knowing the relative level of seeding. To reduce the chances of underestimating the potential density that a particular type of constructed pool could support and to treat constructed pools in the same way that we had treated natural habitats (Nickelson et al. 1992), we eliminated from our analysis those streams that we judged were underseeded (i.e. those having an average density of juvenile coho salmon of less than 1.0 fish/m² in natural pools (see Nickelson et al. 1992)). To insure an adequate number of juvenile coho salmon to seed winter habitat, we resampled the summer pools during the winter only in streams that had an average density during summer of at least 0.7 fish/m² in scour pools (Nickelson et al. 1992). In each stream during summer, we tried to sample at least five natural pools in the vicinity of the constructed pools to determine the relative seeding level.

Sampling techniques

Between 1986 and 1989, we sampled pools constructed by the U.S. Bureau of Land Management, the USDA Forest Service, or the Oregon Department of Fish and Wildlife in 21 coastal Oregon streams (see Nickelson et al. 1992 for a description of a typical study stream). Most of the pools we sampled were created by the construction of structures placed across the full width of the stream channel, the most common technique employed in coastal Oregon streams. Material used for construction of these structures included rock-filled gabions (wire

baskets), logs or timbers, boulders, a combination of boulders and logs, or concrete. Full-width structures were arranged perpendicular to the channel, diagonal to the channel, or in a downstream "V." A second category of habitat improvement that we sampled was constructed alcoves, quiet water areas or ponds excavated into the streambank (similar to the "refuge bays" of Peterson (1985)). We also sampled pools created by a variety of other techniques, such as placement of boulders, log deflectors, and blasting in bedrock.

Sampling took place during the summer low-flow period (August–mid-October) and during winter following a bank-full flow event (December–mid-February). We sampled 21 streams and 199 pools during the summers of 1986–89 and 19 streams and 181 pools during the winters of 1986–89. The pools sampled during winter were a subset of those sampled during summer.

We estimated the population of juvenile coho salmon in each pool by blocking the pool off with seines and conducting a mark–recapture estimate (Chapman 1951) using electrofishing equipment and seines. We estimated the surface area and calculated the density of juvenile coho salmon for each pool. We measured the maximum depth of each pool during winter.

We classified each constructed pool according to type: plunge pool, dammed pool, scour pool, or alcove (Bisson et al. 1982; Nickelson et al. 1992). Typically, pools created upstream of full-width structures were classified as dammed pools whereas those created downstream were classified as plunge pools.

Analysis

We limited our analysis to constructed alcoves and to dammed pools and plunge pools associated with full-width structures (construction materials combined) because these were most common and were the only habitats constructed in more than one stream. We calculated the mean density of coho salmon during summer and winter for constructed plunge pools, dammed pools, and alcoves. We used the Kolmogorov–Smirnov test (Sokal and Rohlf 1969) to test for normality of distributions and Bartlett's test (Snedecor and Cochran 1967; Sokal and Rohlf 1969) to test for homogeneity of variances among pool types. We used a natural logarithm transformation on the habitat-specific densities when necessary to correct for nonnormal distributions or heterogeneous variances.

We used analysis of variance (ANOVA) to compare the means of juvenile coho salmon density among types of constructed pools during summer. We were unable to make similar comparisons among types of constructed pools during winter because the variances among the pool types were heterogeneous and large variance was paired with small sample size despite a natural logarithm transformation (see Day and Quinn 1989).

To determine whether juvenile coho salmon used constructed pools to the same extent that they used natural pools during summer and winter, we made comparisons with data collected for the purpose of describing juvenile coho salmon habitat use (see Nickelson et al. 1992). We have found that juvenile coho salmon show no preference among natural pool types during summer but they do during winter (Nickelson et al. 1992). We therefore used ANOVA to compare the mean density in natural and constructed pools of combined types in summer, but of separate types in winter.

Brush Placement Experiment

Sampling techniques

We noted that pools associated with constructed structures tended to lack the quantity of cover in natural pools of the same

type. We therefore designed an experiment to test whether the placement of bundles of small trees (brush bundles) in constructed pools would increase the winter carrying capacity of those pools for juvenile coho salmon. The experiment consisted of (1) sampling pools created by full-width structures during summer and winter using the methods described above, (2) cabling bundles of two or three small (3–12 m in length) conifer or alder trees (brush bundles) to the streambank of a subset of the pools following a second summer's sampling, and (3) sampling during the winter following brush placement.

The experiment encompassed 24 pools treated with brush bundles and 17 control pools located in seven streams. Twenty of the pools were classified as plunge pools and 21 were classified as dammed pools. Sixteen of the pools were created by rock gabions, nine by log sills, eight by boulder berms, six by concrete sills, and two by combination boulder and log berms.

Analysis

The data from all streams were pooled. Separate comparisons were made for dammed pools, plunge pools, and total pools. Natural logarithm transformations were used to normalize distributions or equalize variances. Comparisons between treatments and controls were made by analysis of covariance (ANCOVA) (Snedecor and Cochran 1967) using pretreatment densities as the covariate. Comparisons were made of the mean winter density and the mean percentage of the summer population remaining during winter. This second variable was used to account for any differences that might have occurred in density between summers and among streams.

Results

Evaluation of Existing Constructed Habitat

Mean densities of juvenile coho salmon varied among constructed habitats and among seasons (Fig. 1). Densities in individual pools ranged from 0.03 to 4.88 fish/m² during summer and from 0.01 to 3.30 fish/m² during winter.

We found no significant difference in mean density of juvenile coho salmon among types of constructed pools during summer (ANOVA, $P = 0.31$). The mean density in the constructed pools during summer was similar to that which we found for natural pools (ANOVA, $P = 0.93$).

Our data suggest (although we were unable to test them statistically) that constructed alcoves support much greater densities of juvenile coho salmon during winter than do either constructed plunge or dammed pools (Fig. 1). Mean density of juvenile coho salmon in constructed plunge pools during winter was similar to density in natural plunge pools (ANOVA on transformed data, $P = 0.98$), and mean density of juvenile coho salmon in constructed alcoves was similar to density in natural alcoves (ANOVA, $P = 0.80$). However, mean density of juvenile coho salmon in constructed dammed pools during winter was significantly less than the mean density in natural dammed pools (ANOVA on transformed data, $P = 0.04$). Six constructed dammed pools had a winter density greater than 1.0 fish/m², but the remaining 47 constructed dammed pools had a winter density less than 0.5 fish/m². The dammed pools with high density had a greater mean maximum depth than did the dammed pools with low density (ANOVA on transformed data, $P = 0.04$).

Brush Placement Experiment

After adjusting for differences in density of coho salmon during the pretreatment year between pools with and without brush,

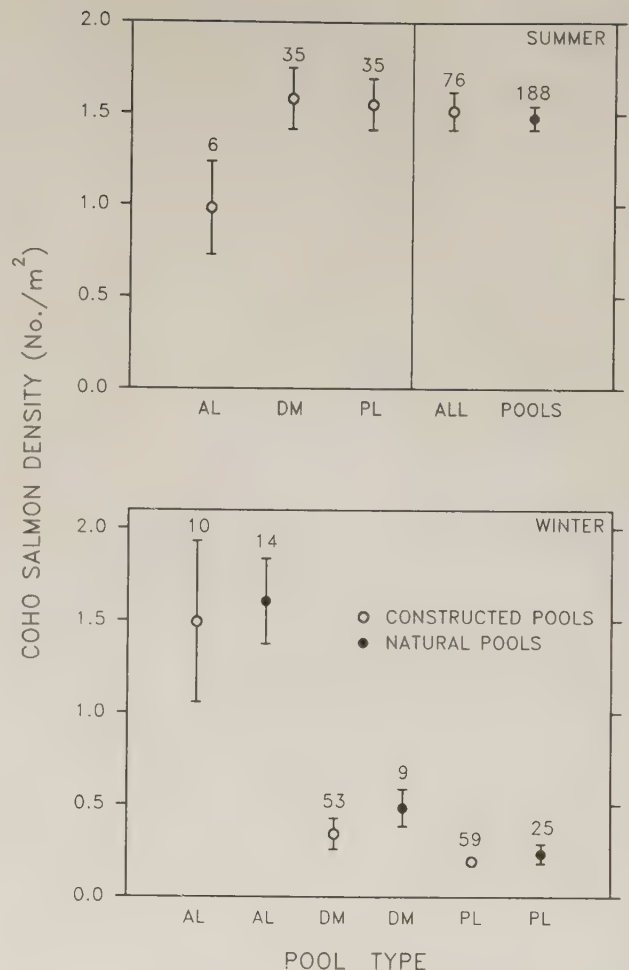


FIG. 1. Mean and standard error for density of juvenile coho salmon in constructed and natural pools during summer and winter. The standard error for constructed plunge pools is smaller than the point for the mean. Sample size is shown above each pool type. AL = alcove; DM = dammed pool; PL = plunge pool.

mean density of coho salmon in pools with brush was significantly greater than in those without (ANCOVA on transformed data, $P = 0.01$, Fig. 2). However, these differences occurred in dammed pools (ANCOVA on transformed data, $P = 0.02$, Fig. 2) but not in plunge pools (ANCOVA on transformed data, $P = 0.33$, Fig. 2). Results of the comparisons using the percentage of the summer population remaining during winter were similar to those with mean density in pools combined, dammed pools, and plunge pools (ANCOVAs on transformed data, $P = 0.01$, $P = 0.047$, and $P = 0.37$, respectively).

The addition of brush increased the mean density of coho salmon in the constructed dammed pools during the winter to a level not significantly different from that of natural dammed pools (ANOVA on transformed data, $P = 0.96$).

Discussion

The construction of full-width structures, the most common habitat improvement technique employed in Oregon coastal streams, has resulted in the creation of habitat suitable for rearing of juvenile coho salmon during summer. Constructed alcoves also provide habitat during summer. During summer, coho salmon prefer pool habitat regardless of type (Nickelson et al. 1992). We found the density of juvenile coho salmon in

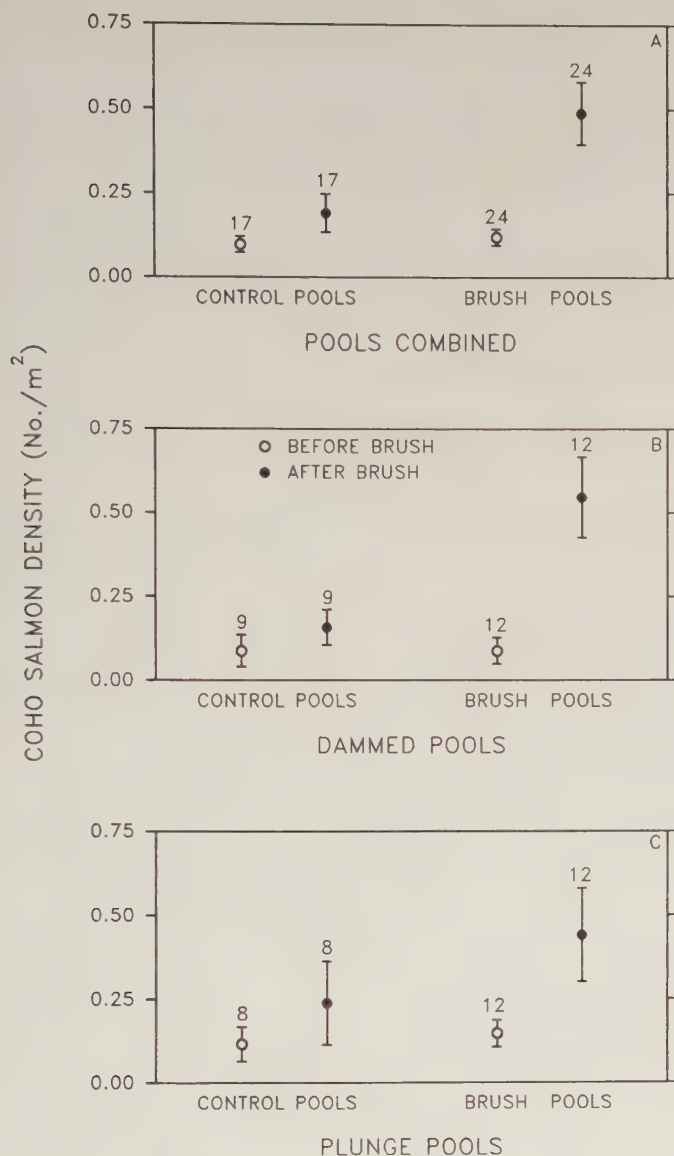


FIG. 2. Mean and standard error for winter density of juvenile coho salmon in constructed pools in the years before and after the addition of brush. The comparisons are between control pools, which received no brush, and brush pools, to which brush was added in the second year, for (A) all pools combined, (B) dammed pools, and (C) plunge pools.

constructed pools during summer to be similar to that of natural pools. House and Boehne (1986) found that full-width structures resulted in an increase in pool area and a threefold increase in number of coho salmon during summer.

However, Nickelson et al. (1992) suggested that, given adequate spawners, winter habitat limits production of coho salmon smolts in most Oregon coastal streams, and with few exceptions (i.e. some deep dammed pools), constructed, full-width structures do not create suitable winter habitat for coho salmon. Throughout their range, juvenile coho salmon prefer low-velocity areas for overwintering (Tschaplinski and Hartman 1983; Hartman and Brown 1987; Cederholm et al. 1988; Shirvell 1990; Nickelson et al. 1992). We were not surprised that plunge pools below full-width structures did not provide winter habitat because natural plunge pools also provide poor winter habitat (Nickelson et al. 1992). Constructed and natural plunge pools in this study supported similar densities of juvenile coho

salmon during winter (Fig. 1). Natural dammed pools provide moderate habitat for juvenile coho salmon during winter, but not as good as alcoves and beaver ponds (Nickelson et al. 1991). However, constructed dammed pools supported a lower density of juvenile coho salmon during winter than did natural dammed pools (Fig. 1). The few constructed dammed pools that supported a high density of juvenile coho salmon (>1 fish/m²) during winter were significantly deeper than those with low densities of salmon. The area above a full-width structure is a natural area of deposition (Reeves and Roelofs 1982), and many full-width structures are installed to collect spawning gravel (House and Boehne 1985, 1986; Klassen and Northcote 1988). Thus, a dammed pool above the structure will often become shallow through time. Our results suggested that dammed pools should be designed to maintain depth if they are constructed to provide winter habitat.

By adding bundles of small trees to constructed dammed pools, we were able to increase the density of coho salmon inhabiting the pools during winter to a level similar to that in natural pools. The brush bundles probably decreased water velocity at high stream flow and provided cover at low stream flow. However, we could detect no effect of adding tree bundles to constructed plunge pools. This may be because plunge pools, by nature, are turbulent, and the brush bundles failed to reduce turbulence and velocity at high stream flow.

Constructed alcoves do provide winter habitat for juvenile coho salmon. We found the density of juvenile coho salmon in constructed alcoves during winter to be greater than that in constructed dammed and plunge pools and similar to that of natural alcoves, a preferred habitat of juvenile coho salmon in Oregon coastal streams (Nickelson et al. 1992). Bustard and Narver (1975) found that juvenile coho salmon during winter showed a preference for constructed "sidepools" over main-channel habitat, particularly at low water temperature and if overhead cover was provided. Peterson (1985) found a low density in a constructed habitat similar to an alcove; however, the seeding level of the stream was apparently also low.

We did not make statistical comparisons of mean density of juvenile coho salmon among constructed habitat types during winter because of the large variance associated with density in man-made alcoves relative to that of the man-made dammed and plunge pools. Most man-made alcoves supported high density of juvenile coho salmon during winter. However, a few supported almost no fish at all. The large variation in density can in large part be explained by the variability in the characteristics of the access channel into the alcove. When the access channel had adequate depth and flow and the entrance to the channel was open, we found the density of juvenile coho salmon to be higher than when the channel was shallow, partially obstructed with debris or sediments, or had low or diffuse flow into the main stream. Peterson (1985) identified the point where the access channel joins the stream as the most critical aspect of alcove design. Extreme care must be taken when designing and constructing alcove habitat to insure that the access channel remains open during all flow levels. This can often be accomplished by locating the alcoves in areas where small intermittent tributaries or springs join the main channel or by adding a full-width structure downstream of the entrance and backing up the stream to keep the entrance flooded.

Natural alcove habitat is rare in Oregon coastal streams, often comprising less than 1% of the total surface area during winter (see table 3 in Nickelson et al. 1992). The positive results we found for man-made alcoves suggest that this habitat type, if

properly constructed, may increase production of juvenile coho salmon in streams where it occurs infrequently.

This study demonstrates that when planning habitat enhancement, the type of habitat preferred by a species and the availability of that habitat throughout the year must be considered. A technique that creates suitable habitat during summer may not create suitable habitat during winter. A technique that creates habitat that imitates the natural habitat preferred by the species of interest and that is in shortest supply will likely be successful in increasing production.

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Regional Differences in Otolith Morphology of the Deep Slope Red Snapper *Etelis carbunculus*

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Smith, M. K. 1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. Can. J. Fish. Aquat. Sci. 49: 795–804.

Sagittal otoliths from four populations of the Pacific deep slope red snapper *Etelis carbunculus* Cuvier were compared using Fourier descriptors and other shape indices, linear proportions, and dry weight. Otoliths from Hawaii, Vanuatu, Fiji and French Polynesia and a small number from the Commonwealth of the Northern Marianas Islands (NMI) were examined. Regional shape and weight characteristics were distinguishable, despite the wide range of individual variation and limited available size range from some regions. Size-specific differences in otolith shape were found for the four regions for which a sufficient sample was available. Otoliths from Hawaii, French Polynesia, and NMI showed a significant shape affinity. Otoliths from Fiji and Vanuatu were similarly shaped and were distinct from those from the other three regions. Interregional otolith shape affinities for the stocks examined parallel similarities in maximum size and growth rate from the literature, suggesting that growth rate may influence otolith shape. Observed trends in otolith weight as a function of fish length support growth-related regional differences in otolith shape.

Des otolithes sagittaux de quatre populations de vivanneaux du Pacifique (*Etelis carbunculus* Cuvier) ont été comparés à l'aide de descripteurs de Fourier et d'autres indices de taille, des proportions linéaires et du poids sec. Des otolithes provenant d'Hawaï, du Vanuatu, des Fidji et de la Polynésie française et un petit nombre du Commonwealth des îles Mariannes du Nord (IMN) ont été examinés. On pouvait distinguer des caractéristiques régionales de forme et de poids, en dépit de la grande diversité de variations individuelles et de la plage limitée des tailles disponibles pour certaines régions. On a observé les différences propres à l'espèce dans la forme des otolithes dans quatre régions pour lesquelles un échantillon suffisant était disponible. Les otolithes d'Hawaï, de la Polynésie française et des IMN présentaient une affinité de forme significative, alors que les otolithes des Fidji et de Vanuatu avaient des formes semblables et étaient distinctes de celles des trois autres régions. Les profils des affinités interrégionales des formes d'otolithes des stocks examinés sont semblables aux ressemblances des tailles maximum et des taux de croissance mentionnées dans la documentation, ce qui suggère que le taux de croissance peut influencer la forme des otolithes. Les tendances observées du poids des otolithes en fonction de la longueur du poisson appuient l'hypothèse des différences régionales en rapport avec la croissance pour expliquer la forme des otolithes.

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The ehu (*Etelis carbunculus*) is a red snapper of the family Lutjanidae. The Etelinae, one of the four subfamilies of Lutjanidae, comprise five genera: *Aphareus*, *Aprion*, *Etelis*, *Pristipomoides*, and *Randallichthys*. *Etelis carbunculus* are captured from the coast of Africa as far east as Hawaii and French Polynesia, as summarized by Allen (1985). They represent one of the principal species in the commercial hand-line catch from steep, rocky slopes at depths of 200–450 m.

Throughout the Pacific, *Etelis carbunculus* Cuvier (in Cuvier and Valenciennes 1828) has been referred to as *Etelis marshi*. Both Fourmanoir (1971) and Anderson (1981) pointed out that there were no significant morphological differences between specimens collected in the Pacific and those from the Seychelles Archipelago, previously described as *Etelis carbunculus*. Anderson's (1981) clarification of the morphological distinctions between *Etelis coruscans* and *Etelis carbunculus* further reduced confusion regarding the

geographic distribution and taxonomy of these two snappers. However, significant questions remain regarding the remarkable differences in the maximum size reached by *Etelis carbunculus* in different areas of the Pacific.

Fishermen and fisheries biologists have brought to light differences of over 50 cm in the maximum size of ehu captured in various regions of the northern and southern tropical Pacific. Fish measuring up to 90–110 cm (fork length) are found between the Cook Islands, Papua New Guinea, and the northeast coast of Australia (Brouard and Grandperrin 1985; Schaan et al. 1987; Lewis et al. 1988; Carlot and Nguyen 1989; Latu and Tulua;² Lokani et al.³; Nath and

²T. F. Latu and S. Tulua. Estimated maximum sustainable yield for Tonga deep bottom fishery. Contrib. Minist. Agric., Fish. and For., Fish. Div., Kingdom of Tonga, USAID Trop. Fish. Res. Assess. Workshop, July 1989, Honolulu, HI. 15 p. (Manuscr.)

³P. Lokani, H. Pili, and G. Tiroba. Assessment of bottomfish resources of Papua New Guinea. USAID Trop. Fish. Res. Assess. Workshop, July 1989, Honolulu, HI. 13 p. (Manuscr.)

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Sesewa⁴; Sua⁵). The largest fish reported in this region, at Vanuatu, weighed 12.2 kg and is estimated to have measured 127 cm (R. Grandperrin, ORSTOM, Centre de Noumea, Boite Postale A5, Noumea Cédex, Nouvelle Calédonie, pers. comm.). Maximum fish sizes for Hawaii and French Polynesia and in the range of 65–75 cm (Uchiyama and Tagami 1984; Wrobel 1985; Ralston and Kawamoto 1987), while ehu as large as 58 cm are rarely found in the region surrounding the Commonwealth of the Northern Mariana Islands (NMI) (Ralston and Williams 1988). Regional differences in maximum size between these stocks are evidence of their relative isolation, probably maintained by the distances and depths that separate them.

This study describes regional differences in otolith morphology in five areas of the Pacific. It began as an effort to evaluate the reasons for differences in maximum size. Regional otolith shape differences were so remarkable that they were first believed to be an indication of taxonomic differences between stocks. However, examination of regional specimens confirmed that all were *Etelis carbunculus*.

Materials and Methods

Fork lengths were recorded and sagittal otoliths collected from *Etelis carbunculus* captured at five sites throughout the Pacific between 100 and 400 m of depth. Otoliths were washed in 70% alcohol, dried, and stored in plastic envelopes. Following the measurements described below, sagittae were oven-dried and desiccated to a constant weight. A resin cast of the saccular lumen was made for a few fish of each size from Hawaii. Otoliths collected on a regional basis are summarized below:

Region	Year(s)	No. of otolith Pairs
Hawaiian Islands	1989	130
Fijian Islands	1987	26
	1989	109
French Polynesia	1985–86	42
	1989	32
NMI	1982	6
	1989	6
Vanuatu	1983	84

The growth focus is a useful point of reference, visible from the unsectioned otolith's concave lateral side. The view of the otolith from this side is referred to herein as the "lateral view" (see Fig. 1). To make the focus more apparent, otoliths were cleared using a glycerine and alcohol (2:3) mixture. The following proportions (Fig. 1) were recorded through a dissecting microscope from the video-relayed image (lateral view) of one of each pair of otoliths, using an optical software system designed by Biosonics, Inc. (1987): (1) distance from tip of rostrum to focus, (2) distance from postrostrum to focus, (3) distance from sulcus groove to tip of rostrum, (4) dorsal (i) and ventral (ii) width at the focus, (5) length from postrostrum to sulcus, (6) length from sulcus to tip of antirostrum, and (7) basal width of antirostrum.

⁴G. Nath and A. Sesewa. Progress report on fisheries research conducted on bottomfish stocks around the Fijian Republic. Contrib. Fish. Div., Minist. Primary Ind., Republic of Fiji, USAID Trop. Fish. Res. Assess. Workshop, July 1989, Honolulu, HI. 21 p. (Manuscr.)

⁵T. Sua. Assessment of bottomfish resources of Western Samoa: an interim report. USAID Trop. Fish. Res. Assess. Workshop, July 1989, Honolulu, HI. 20 p. (Manuscr.)

Otoliths were immobilized parallel to the field of view on a stand of polyurethane foam. The perimeter of each otolith was traced in a counterclockwise direction from two different perspectives, the ventral and lateral views. Each outline was digitized into a set of 128 virtual x, y coordinates, taken at equally spaced intervals along its perimeter, and a Fast Fourier Transform (FFT) of these points was taken of the form

$$(1) \quad R(\Theta) = A_0 + \sum_{n=1}^{128} A_n \cos(\Theta_n + \phi_n)$$

where the original particle representation and angular position ($R(\Theta)$) are decomposed into a standardized starting position (A_0) and 128 consecutive amplitude coefficients (A_n), with angular components (Θ_n) and slope components (ϕ_n). Rotational and size invariance was achieved by (1) dividing the amplitude coefficients by the amplitude of the first descriptor (A_1), (2) setting the A_0 frequency component to zero, and (3) iteratively rotating the shape in the frequency domain until an optimization function was maximized. The technique is outlined briefly in the Biosonics, Inc. (1987) manual and in more detail in references dealing with shape analysis (Alt 1962; Hu 1962; Clark 1981).

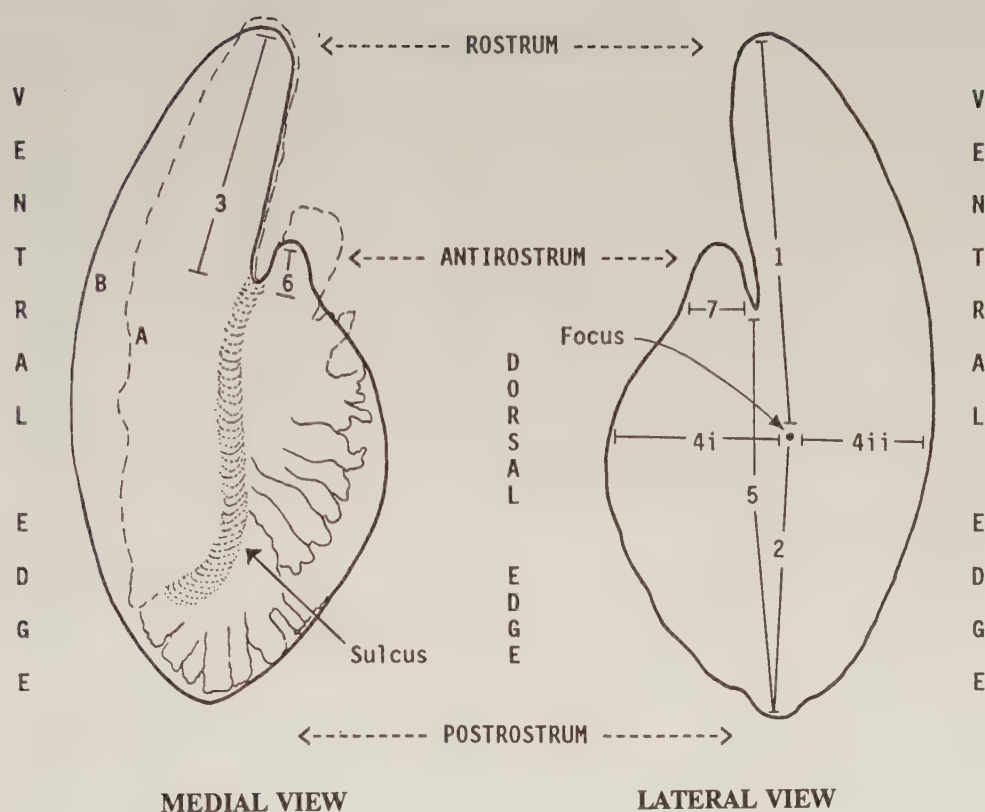
While the video-flattened lateral and ventral images made shape comparisons essentially two-dimensional, the ventral view provided an index of otolith curvature and the lateral silhouette showed much of the variation in overall shape. Two other characteristics recorded from the lateral view were the indices of (1) circularity, the perimeter squared divided by the area, and (2) rectangularity, the shape's area divided by the area of its minimum enclosing rectangle. Normalized Fourier descriptors, scaled indices of rectangularity and circularity, and ratios of linear proportions provided size-independent indices of otolith shape, allowing comparison of different sized otoliths.

The first 20 amplitudes ("harmonics") after A_0 and A_1 were used to characterize each FFT (normalization functions set $A_0 = 0$ and $A_1 = \text{unity}$ for all FFTs). The amplitude of each harmonic (A_n of equation 1) was calculated from Biosonics sine and cosine output (Castonguay et al. 1991) according to the relationship (Christopher and Waters 1974)

$$(2) \quad A_n = \sqrt{\sin_n^2 + \cos_n^2}.$$

To evaluate whether otolith shape indices and weights varied as a function of fish size, otoliths were grouped by 10-cm fork length intervals and the indices were compared between size groups and regions using a Student–Newman–Keuls (SNK) means test (Sokal and Rohlf 1969). Regional nonhierarchical cluster analyses of linear proportions, harmonics, and shape indices were performed by the SAS "Fastclus" procedure (SAS Institute Inc. 1985), which clusters data into discrete groups by nearest centroid sorting using a k -means method (Anderberg 1973) on squared Euclidean distances (referred to hereinafter as NC cluster analysis).

Linear combinations of the multivariate regional shape characteristics were compared by canonical discriminant analyses (SAS "Candisc" procedure). The first and second canonical variables are maximally uncorrelated descriptors of the first- and second-order multiple regressions, respectively, used to characterize the sample in two dimensions. Interregional groupings were also evaluated using hierarchical average linkage cluster analyses (SAS "Cluster" procedure) on squared Euclidean distances. To avoid the disproportionate weighting of measurements made in different units or showing a wider range of variation, all cluster analyses were performed on data mat-



GENERAL SHAPE SHOWN FOR THE FOLLOWING REGIONS (MEDIAL VIEW)

A Fiji/Vanuatu

B Hawaii/NMI/French Polynesia

Symbol

----- (dashed line)

———— (solid line)

FIG. 1. Medial and lateral views of the right sagittal otolith of the deep slope snapper (*Etelis carbunculus*).

rices standardized to dimensionless units with mean zero and standard deviation unity (Romesburg 1984).

Results

Dissection of fish skulls showed that the otolith abuts against the ventral and medial wall of each otic capsule (sacculus). Both the dorsal edge and outer lateral surface of the otolith are unconstrained by the bony walls of the otic capsule, extending into the macular lumen and held in place only by a gelatinous material. Resin casts of the saccular lumen for fish from Hawaii produced a mold almost identical to the ventral and medial surfaces of the otolith, while the casts were 4–6 times as thick and concave rather than convex like the otolith on their outer surface. Otoliths of small fish (<30 cm fork length) had some space within the otic capsule, even along the medial surface, while otoliths of medium-sized (30–50 cm) and large (>50 cm) fish fit more and more snugly into the sacculus, their shape increasingly molded to that of the ventral and medial surfaces of the otic capsule. The constraints on otolith shape imposed by the chamber that contains them may account for

much of the curvature and uniqueness of form which develops in the otoliths of large fish.

The relationship between fork length and linear otolith dimensions was examined by log-linear regression (Table 1). Although results obtained by this method are affected by the size range included in each regression, they are mentioned because of the interesting variation observed for all regions. Regression parameters for all six linear proportions (illustrated in Fig. 1) were allometric (b always less than 1) and varied in a regional and character-specific manner. While differences between regional samples are to be expected for several reasons, within-region variation in otolith growth rate along different axes (evidenced by within-region variation in b values) and in individual variability of these traits for the same otoliths (indicated by varying coefficients of determination, r^2) illustrates the complex three-dimensional nature of otolith growth. Regional regression parameters for otolith weight versus fork length are included in Table 1, along with predicted values of otolith weight within an intermediate fork length range, for the four regions that were well represented numerically. Predicted weight for Vanuatu was the lowest, followed in increasing order by Fiji, Hawaii, and French Polynesia.

TABLE 1. Allometric relationships for fork length versus otolith weights and linear dimensions.

Region	Otolith weight/ linear dimension	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i> ²
Hawaii	Otolith weight	4.383×10^{-6}	1.66	106	0.9512
	Rostral radius	222.2	0.56	128	0.7605
	Postrostral radius	165.6	0.58	128	0.8323
	Total length (at focus)	387.9	0.57	128	0.8949
	Dorsal radius	140.2	0.52	128	0.8134
	Total width (at focus)	217.4	0.55	128	0.8798
	Postrostrum-sulcus length	479.3	0.48	128	0.8573
NMI	Otolith weight	3.038×10^{-6}	1.74	9	0.8611
	Rostral radius	65.1	0.78	12	0.8193
	Postrostral radius	282.1	0.50	12	0.8511
	Total length (at focus)	255.6	0.65	12	0.8578
	Dorsal radius	443.4	0.32	12	0.3419
	Total width (at focus)	417.9	0.44	12	0.6415
	Postrostrum-sulcus length	388.1	0.52	12	0.7647
French Polynesia	Otolith weight	4.193×10^{-7}	2.10	67	0.9038
	Rostral radius	40.6	0.86	69	0.8464
	Postrostral radius	132.3	0.63	69	0.6510
	Total length (at focus)	138.2	0.76	69	0.8713
	Dorsal radius	80.0	0.63	69	0.6517
	Total width (at focus)	146.7	0.62	69	0.7608
	Postrostrum-sulcus length	477.3	0.49	69	0.7467
Fiji	Otolith weight	3.784×10^{-7}	2.017	105	0.8250
	Rostral radius	110.9	0.68	108	0.7774
	Postrostral radius	70.0	0.73	108	0.7510
	Total length (at focus)	179.2	0.70	108	0.8264
	Dorsal radius	88.6	0.58	108	0.4880
	Total width (at focus)	227.6	0.52	108	0.6381
	Postrostrum-Sulcus length	278.0	0.58	108	0.6921
Vanuatu	Otolith weight	5.164×10^{-6}	1.60	82	0.8859
	Rostral radius	204.1	0.59	84	0.8718
	Postrostral radius	101.7	0.70	84	0.8895
	Total length (at focus)	295.7	0.63	84	0.9158
	Dorsal radius	188.3	0.48	84	0.7345
	Total width (at focus)	332.9	0.47	84	0.8081
	Postrostrum-sulcus length	449.3	0.51	84	0.8682

Equation: $y = ax^b$ where x = fork length (mm) and y = linear dimension (μm) or dry weight (g)

Fork length (mm)	Predicted otolith weight (g)			
	Fiji	Vanuatu	Hawaii	French Polynesia
300	0.038	0.048	0.057	0.063
400	0.067	0.077	0.092	0.114
500	0.105	0.110	0.133	0.182
600	0.152	0.147	0.180	0.267
700	0.208	0.188	0.233	0.368

Regional differences in otolith morphology are a complex topic, since otoliths from all regions (except NMI for which the size range and number of samples were too small) showed significant differences in shape as a function of size. Table 2 summarizes the results of SNK means comparisons for each region for indices of otolith circularity and rectangularity. A circle would have the minimum circularity value ($4\pi = 12.57$), while a rectangle (or square) would have the maximum rectangularity value (unity). Tabled values indicate that large otoliths from all regions were both less circular and less rectangular than smaller ones. In some cases, decreasing circularity or rectangularity was apparent, but not statistically significant, due to a high sample-wide variability in shape and/or a small number of samples in some size groups. It is worth noting that the indices of circularity and rectangularity are not inverses, since

a circle and a square (or rectangle) have some symmetrical similarities. Furthermore, a shape's departure from rectangularity or circularity varies in two dimensions, while the numerical values of both shape indices vary linearly. This influences each index somewhat differently. Having an equal and larger number of otoliths from each size group would have helped clarify size-related shape differences, but the constraints of fishing and sampling opportunities worked out through cooperative long-distance agreements made this optimum difficult to achieve.

The ratios of linear measurements (Table 3) provided a good index of regional otolith shape variation. Data were pooled irrespective of size because they showed the same regional groupings (SNK, $\alpha \leq 0.01$), regardless of whether they were segregated by size. Regional groupings were similar to those seen for indices of circularity and rectangularity. The long and nar-

TABLE 2. Results of SNK means comparisons ($\alpha = 0.01$) for average otolith circularity and rectangularity as a function of size group by 10-cm intervals of fork length. Underlined values are not significantly different.

Region/characteristic	Length group (cm)						
	<30	31-40	41-50	51-60	61-70	71-80	>80
Mean circularity	← (more circular)						
Hawaii	23.45	<u>27.06</u>	<u>28.64</u>	31.13	<u>31.28</u>		
French Polynesia	<u>24.24</u>	<u>25.74</u>	<u>28.53</u>	36.08			
Fiji			<u>31.94</u>	<u>35.28</u>	<u>38.80</u>	<u>45.87</u>	<u>46.71</u>
Vanuatu	<u>29.30</u>	<u>29.00</u>	<u>33.89</u>	<u>33.86</u>	<u>39.75</u>	<u>45.47</u>	<u>42.98</u>
NMI	<u>26.32</u>	<u>27.97</u>	<u>29.97</u>				
Mean rectangularity	← (more rectangular)						
Hawaii	<u>0.6372</u>	<u>0.6202</u>	<u>0.6064</u>	<u>0.5966</u>	0.5381		
French Polynesia	<u>0.6453</u>	<u>0.6207</u>	<u>0.6122</u>	<u>0.5850</u>			
Fiji			0.5726	<u>0.5251</u>	<u>0.5044</u>	<u>0.4999</u>	<u>0.4865</u>
Vanuatu	<u>0.6003</u>	<u>0.5940</u>	<u>0.5369</u>	<u>0.5304</u>	<u>0.5238</u>	<u>0.4924</u>	<u>0.4791</u>
NMI	<u>0.6455</u>	<u>0.6468</u>	<u>0.6439</u>				
Number of otoliths per size group							
Hawaii	42	28	37	11	1		
French Polynesia	17	40	10	2			
Fiji			8	35	33	19	13
Vanuatu	4	11	12	8	19	23	7
NMI	5	5	2				

TABLE 3. Results of SNK comparisons ($\alpha = 0.05$) between mean ratios of linear otolith measurements. Underlined values are not significantly different.

Ratios of otolith linear measurements	Regional mean (%)				
	Fiji	Vanuatu	NMI	Hawaii	French Polynesia
Rostral radius/Total length	52.47	<u>53.76</u>	<u>54.10</u>	<u>54.18</u>	<u>54.76</u>
Postrostral radius/total length	47.53	<u>46.24</u>	<u>45.90</u>	<u>45.82</u>	<u>45.24</u>
Postrostral radius/postrostrum-sulcus length	67.59	65.48	<u>63.45</u>	<u>62.75</u>	<u>61.63</u>
Total width/total length	39.65	41.66	<u>50.21</u>	<u>49.55</u>	<u>49.39</u>
Total width/postrostrum-sulcus length	56.42	58.88	<u>69.47</u>	<u>67.92</u>	<u>67.27</u>
Dorsal width/postrostrum-sulcus length	31.98	34.48	<u>37.59</u>	<u>37.36</u>	<u>37.00</u>
n for all tests	108	84	12	128	69

row otoliths from Fiji and Vanuatu segregated from those from the other regions, except for two instances where Vanuatu was grouped with the other three locations. Otoliths from Fiji and Vanuatu were significantly different for these proportions, while those from French Polynesia, Hawaii, and NMI could not be distinguished statistically.

Using the same six linear ratios and a seventh character, the ratio of the length from focus to tip of rostrum to that from postrostrum to sulcus (1:5 from Fig. 1), NC cluster analysis produced similar regional groupings (Table 4). The five-cluster-level partitioning of regional samples can be compared visually (tabled percentages in the last row of each section). Hawaiian and French Polynesian samples were primarily

represented in clusters 1 and 3 whereas otoliths from Fiji and Vanuatu were more abundant in clusters 4 and 5. The sample size for NMI was very small, but these otoliths were mostly grouped into clusters 1 and 3 with those from Hawaii and French Polynesia.

The A_{2-22} harmonics (ventral and lateral views) formed similar regional NC clusters (Table 5). Since the results were consistent, regardless of size category, data were again pooled. Whatever index of shape was chosen, otoliths from Fiji and Vanuatu were segregated from those from Hawaii, French Polynesia, and NMI. As might be expected, FFT shapes produced more discrete clusters than linear proportions, and these were more well defined for the lateral than for the ventral view.

TABLE 4. Regional nearest centroids clusters of the ratios of seven otolith traits for deep slope red snapper. Data presented as frequency, overall %, row %, and column %.

Cluster	Region					No. per cluster and %
	Fiji	Hawaii	NMI	French Polynesia	Vanuatu	
1	1	71	6	35	6	119
	0.25	17.71	1.50	8.73	1.50	29.68
	0.84	59.66	5.04	29.41	5.04	
	0.93	55.47	50.00	50.72	7.14	
2	6	3		2	3	14
	1.50	0.75		0.50	0.75	3.49
	42.86	21.43		14.29	21.43	
	5.56	2.34		2.90	3.57	
3	3	37	4	20	2	66
	0.75	9.23	1.00	4.99	0.50	16.46
	4.55	56.06	6.06	30.30	3.03	
	2.78	28.91	33.33	28.99	2.38	
4	48	2	1		20	71
	11.97	0.50	0.25		4.99	17.71
	67.61	2.82	1.41		28.17	
	44.44	1.56	8.33		23.81	
5	50	15	1	12	53	131
	12.47	3.74	0.25	2.99	13.22	32.67
	38.17	11.45	0.76	9.16	40.46	
	46.30	11.72	8.33	17.39	63.10	
Total <i>n</i>	108	128	12	69	84	401
%	26.93	31.92	2.99	17.21	20.95	100.00

Pseudo *F* statistic = 151.8

$\chi^2_{(df\ 24, P < 0.0001)} = 632.9$

Ratios of otolith linear proportions	Corresponding numerical references from Fig. 1
Rostral radius/total length	1:(1 + 2)
Rostral radius/postrostrum-sulcus length	1:5
Postrostral radius/total length	2:(1 + 2)
Postrostral radius/postrostrum-sulcus length	2:5
Total width/total length	(4i + 4ii):(1 + 2)
Total width/postrostrum-sulcus length	(4i + 4ii):5
Dorsal width/postrostrum-sulcus length	4i:5

Canonical discriminant analyses (CDA) and hierarchical clustering were designed to evaluate between-region shape affinities. CDA were used to represent multidimensional shape vectors in a single plane, allowing both quantitative and qualitative assessment of the proximity and content of specific clusters. Plots of canonical discriminant functions from both types of shape indices (Fig. 2) show two principal clusters connected by a transition zone. The cluster group nearest the origin and the y-axis primarily contains otoliths from Hawaii and French Polynesia (with a few from NMI). The transition zone and the cluster closer to the upper range of the x-axis (and widely dispersed along the y-axis) are mainly composed of otoliths from Fiji and Vanuatu. The results are shown for six linear dimensions, circularity, and rectangularity (Fig. 2A) and for the first 10 harmonic descriptors (Fig. 2B, lateral view).

Using regional mean values for the three sets of traits listed in Tables 4 and 5, hierarchical cluster analyses linked otolith shapes from French Polynesia, NMI, and Hawaii and distinguished them from those from Vanuatu and Fiji (Fig. 3). FFT data show a greater definition between regions, since harmonics represent a more sensitive shape indicator.

TABLE 5. Regional groups by nearest centroid clustering of 20 otolith FFT harmonic amplitudes (A_{2-22}). Data presented as frequency, overall %, row %, and column %.

Cluster	Region					No. per cluster and %
	Fiji	Hawaii	NMI	French Polynesia	Vanuatu	
<i>Lateral view</i>						
1	5				3	8
	1.32				0.79	2.11
	62.50				37.50	
	4.85				3.16	
2	21				20	41
	5.53				5.26	10.79
	51.22				48.78	
	20.39				21.05	
3	56	24	2	5	44	131
	14.74	6.32	0.53	1.32	11.58	34.47
	42.75	18.32	1.53	3.82	33.59	
	54.37	18.60	16.67	12.20	46.32	
4	12				7	19
	3.16				1.84	5.00
	63.16				36.84	
	11.65				7.37	
5	9	105	10	36	21	181
	2.37	27.63	2.63	9.47	5.53	47.63
	4.97	58.01	5.52	19.89	11.60	
	8.74	81.40	83.33	87.80	22.11	
Total <i>n</i>	103	129	12	41	95	380
%	27.11	33.95	3.16	10.79	25.00	100.00

Pseudo *F* statistic = 201.9

$\chi^2_{(df\ 24, P < 0.0001)} = 778.5$

<i>Ventral view</i>						
1	35	9	1	2	28	75
	9.19	2.36	0.26	0.52	7.35	19.69
	46.67	12.00	1.33	2.67	37.33	
	33.65	7.03	8.33	4.76	29.47	
2		1				1
		0.26				0.26
		10.000				
		0.78				
3		4		2		6
		1.05		0.52		1.57
		66.67		33.33		
		3.12	4.76			
4	63	44	3	9	58	177
	16.54	11.55	0.79	2.36	15.22	46.46
	35.59	24.86	1.69	5.08	32.77	
	60.58	34.38	25.00	21.43	61.05	
5	6	70	8	29	9	122
	1.57	18.37	2.10	7.61	2.36	32.02
	4.92	57.38	6.56	23.77	7.38	
	5.77	54.69	66.67	69.05	9.47	
Total <i>n</i>	104	128	12	42	95	381
%	27.30	33.60	3.15	11.02	24.93	100.00

Pseudo *F* statistic = 250.4

$\chi^2_{(df\ 24, P < 0.0001)} = 653.9$

Discussion

This study summarizes evidence of regional and local variation in morphology of the otoliths of *Etelis carbunculus*. Rapid

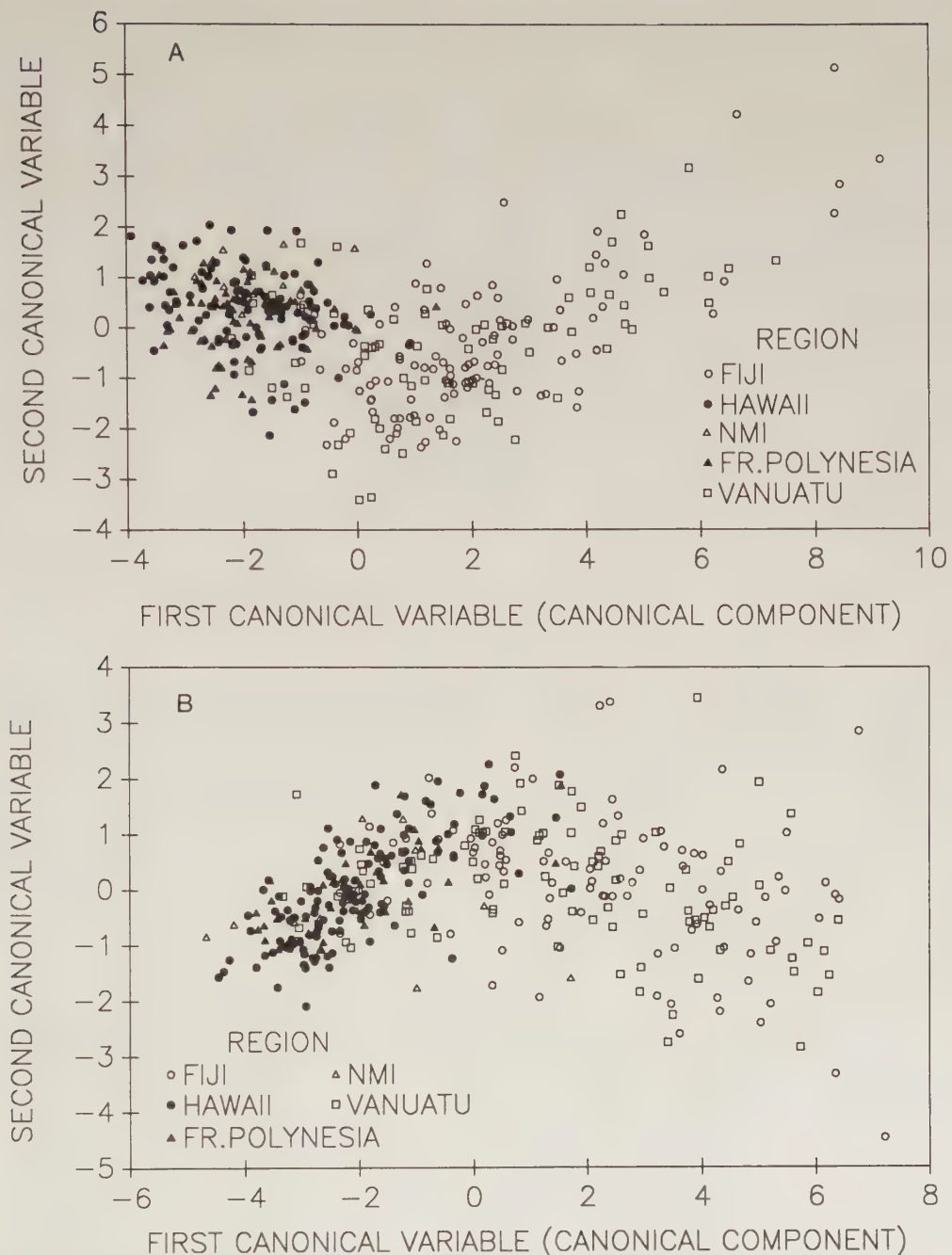


FIG. 2. Plot of canonical discriminant functions from (A) seven linear dimensions, circularity, and rectangularity and (B) 10 FFT harmonics (lateral view) for otoliths of the red snapper.

and localized declines in abundance of this species throughout the Pacific in response to fishing pressure (Schaan et al. 1987; Ralston and Kawamoto 1987; Carlot and Cillaurren⁶; Latu and Tulua (see footnote 2); Nath and Sesewa (see footnote 4)) are an indication of low rates of migration and relatively slow growth which, together with the distance and depth between suitable habitat, lend themselves to the development of regional variation.

Regional differences in growth rate may be a primary factor in the development of differences in otolith shape. The simi-

larity in shape of otoliths from such widely separated regions as NMI, French Polynesia, and the Hawaiian Islands suggests that growth rate represents an important component of otolith shape. These regions have slow or limited growth in common and had thicker, heavier, and more rounded otoliths. Available estimates (Uchida et al. 1982; Brouard et al. 1983; Brouard and Gradperrin 1985; Ralston and Kawamoto 1987; Ralston and Williams 1988; Carlot and Nguyen 1989) indicate that ehu from the southwestern Pacific grow considerably faster than those from Hawaii, NMI, and French Polynesia. Fish from NMI may initially grow at a similar rate to those from the southwestern region (Smith and Kostlan 1991), but do not reach comparably large sizes. Differences in otolith shape may be mediated through differences in the orientation and packing in

⁶A. H. Carlot and E. Cillaurren. Present status in yield assessment for deep bottom fishery in Vanuatu. Contrib. Fish. Dep. and ORSTOM, Port Vila, Vanuatu, USAID/NMFS Trop. Fish. Res. Assess. Workshop, July 1989, Honolulu, HI. 17 p. (Manuscr.)

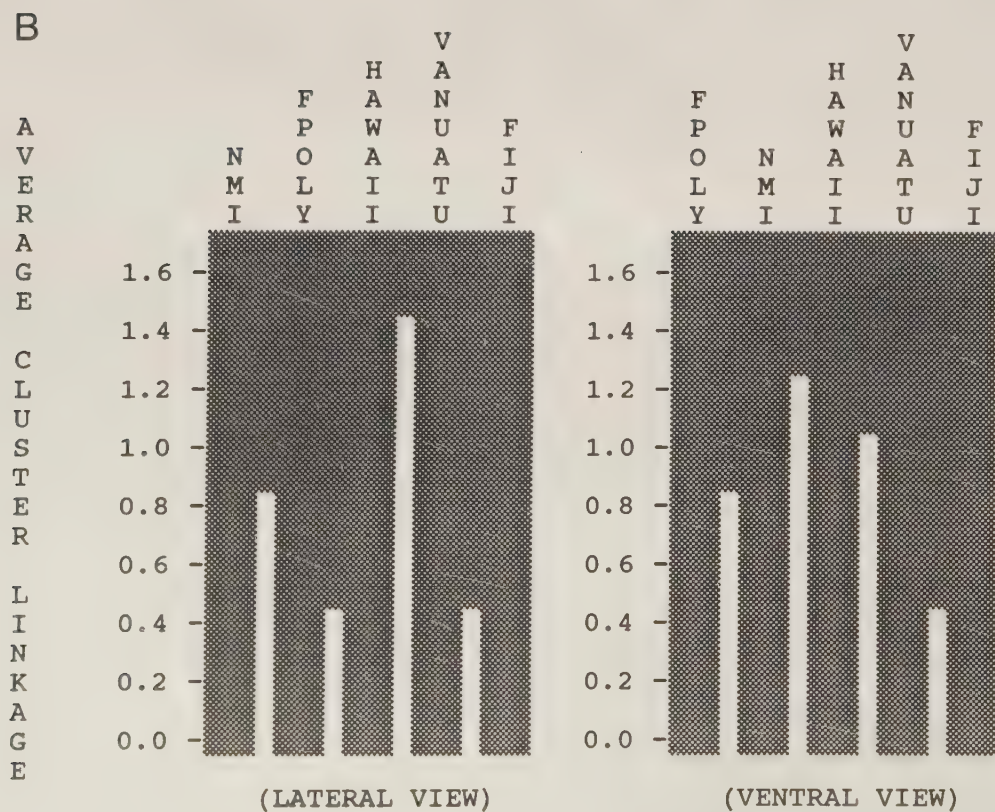
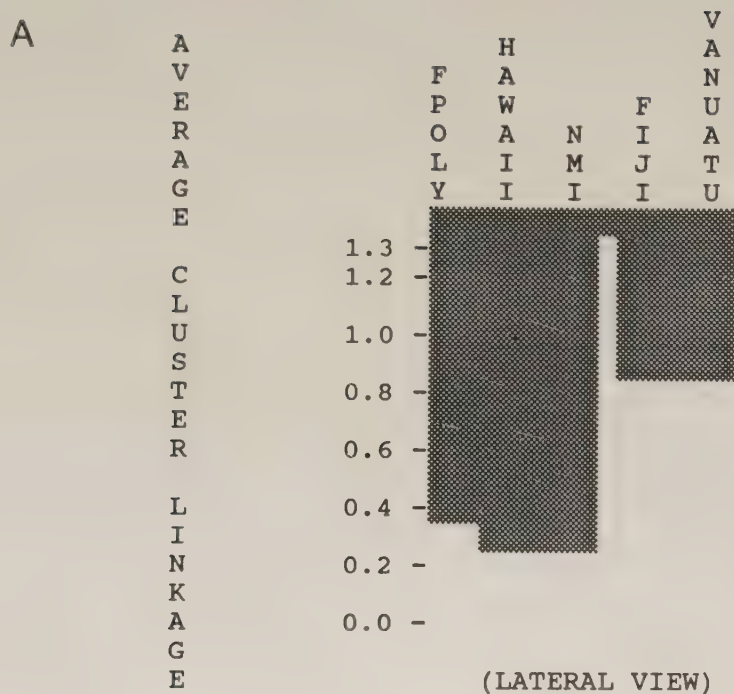


FIG. 3. Hierarchical clusters (cluster dendograms in the style of Johnson 1967) of regional otolith samples based on (A) six linear proportions, circularity, and rectangularity and (B) 20 harmonics.

the otolith crystal, which could easily be influenced by the rate of crystalline growth.

Growth-related differences in linear otolith proportions have been described for other species (Reznick et al. 1989; Secor and Dean 1989). Gaemers and Crajon de Crajon (1986) referred to a sexual dimorphism in otoliths of haplochromines. Faster growing males of these cichlids have more elongate and thinner otoliths. Gauldie et al.⁷ described differences in otolith shape as a function of parasite load for orange roughy (*Hoplostethus atlanticus*). Slow-growing parasitized fish had thicker and wider otoliths. Wilson (1985), describing compensatory depth-related differences in otolith shape for macrourids from the Atlantic and Pacific oceans, found that otoliths were either long and thin or short, wide, and thick. The shape differences noted by Wilson may be attributable to regional and depth-related differences in temperature and/or fish growth rates.

Data compiled by the National Oceanographic Data Center (NODC) (1989) show that mean temperatures at 150–400 m, where ehu are found, are 2–6° lower for Hawaii and NMI than for Fiji and Vanuatu. Temperatures for French Polynesia were 2–3° lower between 275 and 400 m, but were comparable with Fiji and Vanuatu above that depth. Uncoupling of somatic and otolith growth rates as a function of temperature has been described in the literature (Mosegaard et al. 1988). Whether differences in temperature affect otolith shapes directly or through temperature-related constraints on somatic growth cannot be determined from this study. Other factors (such as differences in feeding and food availability between locations) may also be responsible for regional variation in size. Information on habitat, population biology, and morphology of *Etelis carbunculus* must eventually be tied together to evaluate the causes of regional size and shape differences in this species.

Ehu provide an illustration of the continuous changes in shape taking place in otoliths throughout the life cycle. Changes in otolith shape in larval fishes are well known, but post-juvenile shape differences are rarely mentioned in the literature. These changes may be related to shifts in the metabolic rate (Gauldie 1990) as fish mature.

This summary of otolith measurements in several dimensions makes it clear that the otolith does not grow at a constant rate along all axes. For this reason, several planes of sectioning must be considered before attempting to interpret growth from seasonal, annual, or daily growth marks obtained from sections of the otolith (Radtke 1987). Due to the changing relationship between fish age (or size) and otolith shape, regressions of fish length versus linear otolith measurements in any dimension will vary as a function of the size range and number of fish from which data are obtained. The complexity of considerations to be made in selecting a representative size range and number of samples for a given population is illustrated by the fact that the foregoing comparisons between fish of different sizes were affected by size-specific differences in otolith shape, despite the fact that shape indices were scaled to size. Thus, the importance of careful planning to collect a representative sample for the determination of growth parameters must be stressed.

Otolith morphology has long been used in stock differentiation (Einarsson 1951; Zijlstra 1958; Kotthaus 1961; Messieh 1972; Postuma 1974), but recently, otoliths have become

increasingly popular for their timekeeping properties. As Fourier series (Bird et al. 1986; Gauldie and Nelson 1990; Castonguay et al. 1991), scanning electron microscopy (Davies et al. 1988; Gauldie et al. 1990), and other methods make it increasingly feasible to discern differences in otolith shape and molecular structure, researchers have begun to point out a wide range of inter- and intrapopulational differences in otolith shape. Differences in the morphology of otoliths of *Etelis carbunculus* were first believed to be an indication of taxonomic differences in stocks. However, while regional stocks fit the species description, otolith morphology proved to be quite varied and complex. The three-dimensional variation in otolith growth rate described herein represents another indication that growth estimation from single-plane sectioning may someday be viewed as an oversimplification. Since a better understanding of the determinants of otolith structure is paramount to the interpretation of age and growth in fishes, as well as to discussions of stock differentiation and otolith taxonomy, these findings may eventually have more far-reaching applications.

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Body Size and the Ontogeny of the Functional Response in Fishes

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The development of foraging abilities is crucial to the survival and subsequent recruitment of young fishes. We examined experimentally the notion that the foraging abilities of species are so different that useful generalizations across taxa are impossible. We investigated the ontogeny of feeding, reflected in their functional responses, in three Great Lakes' fishes, alewife (*Alosa pseudoharengus*), yellow perch (*Perca flavescens*), and bloater (*Coregonus hoyi*). No strong evidence of species-specific differences in the ontogeny of feeding ability was found. A single size-based relationship explained 52–88% of the variation in the parameters of the functional response equation. We conclude that interspecific differences in feeding abilities of larval fishes may have been over-emphasized, and we suggest that interspecific differences should only be addressed within a size-based framework. This approach appears to provide an acceptable basis for first-order predictions of foraging abilities across taxa, for the identification of exceptional abilities which may lead to advances in the understanding of foraging ability, and for estimating foraging rates for important species for which data are now lacking.

Le développement de l'aptitude au broutage est extrêmement important pour la survie et le recrutement subséquent des jeunes poissons. Nous avons testé la notion selon laquelle les aptitudes au broutage des espèces sont si différentes qu'il est impossible de faire des généralisations utiles d'un taxon à l'autre. Nous avons étudié l'ontogénie de l'alimentation, reflétée par les réponses fonctionnelles de trois poissons des Grands Lacs, la gaspareau (*Alosa pseudoharengus*), la perchaude (*Perca flavescens*) et le cisco de fumage (*Coregonus hoyi*). Très peu d'observations permettent de croire qu'il existe des différences propres aux espèces dans l'ontogénie de l'aptitude à s'alimenter. Un seul rapport basé sur les tailles expliquait 52–88 % des variations des paramètres de l'équation de la réponse fonctionnelle. Nous concluons qu'on a pu accorder trop d'attention aux différences interspécifiques dans les aptitudes à s'alimenter des larves de poissons, et nous suggérons qu'on ne devrait étudier les différences interspécifiques qu'en fonction des tailles. Cette approche semble fournir une base acceptable pour des prévisions du premier ordre des aptitudes au broutage chez les taxons, pour l'identification d'aptitudes exceptionnelles rendant possibles des progrès dans la compréhension de l'aptitude au broutage et pour évaluer les taux de broutage des espèces importantes, pour lesquelles nous ne disposons pas d'un volume suffisant de données.

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A common view in the literature is that the mechanisms controlling recruitment will be specific to individual species because larval survival and subsequent recruitment depends upon a species' unique biological response to its biotic and abiotic environment (Lasker 1987, p. 9). There are important implications for fisheries ecology if we must consider species in isolation. If, for example, the feeding abilities of species are unique, then the detailed information gathered to date about the foraging abilities of many species contributes little to making predictions regarding foraging in an unstudied species. Clearly, species differ in many aspects of their biology. Yet, it is not clear whether species-specific information must always be included or whether general relationships will suffice to explain patterns in the ontogeny of larval and juvenile fishes. Must we begin anew every time we study a different species? Or can we develop expectations for the foraging abilities of an unstudied species from the data available on other species?

Recently, larval size has been suggested as a scale around which to organize information on the early life history of fishes. Miller et al. (1988) investigated the association between fish length throughout the larval period and several key early life history features. Their results indicate that, across species, fish that are larger at hatching are also capable of feeding earlier, have a longer period before reaching irreversible starvation or point of no return, and take longer to absorb their yolk sacs than those hatching at smaller sizes. Furthermore, after hatching, larger fish can generally swim faster (Hunter 1981; Blaxter 1986; Miller et al. 1988), see further (Breck and Gitter 1983; Blaxter 1986), and may be less vulnerable to predation than smaller fish (Brownell 1985; Folkvord and Hunter 1986; Pepin et al. 1987; Miller et al. 1988; Fuiman 1989). Importantly, similarly sized fish appear to have similar abilities, regardless of species or the size at which they hatched (Miller et al. 1988).

The importance of foraging ability in regulating the growth rate and survival rate of young fish is widely recognized (Houde 1987; Persson 1989). The foraging abilities of fish change dramatically between hatching and metamorphosis (Werner and Gilliam 1984). In fact, many species do not have fully devel-

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oped sensory or digestive systems until sometime after hatching (Blaxter 1986; Govoni et al. 1986; Mark et al. 1989). After first feeding, individual fish improve their foraging efficiency rapidly as they develop (Braum 1967; Rosenthal and Hempel 1970; Houde and Schekter 1980; Drost and van den Boogaart 1986; Wanzenböck and Schiemer 1989). These changes in foraging ability are expressed as increased larval growth rates, which, in turn, are significant determinants of larval survival (Houde 1987; Luecke et al. 1990; Rice et al., unpubl. data).

The goals of this paper are to examine to what extent considerations of body size can explain the foraging abilities of three species of fish that hatch at different sizes. We used Holling's type II functional response as an index of feeding ability, which describes the relationship between the number of prey ingested and the prey concentration for each species. This equation is appropriate in situations where a predator is exploiting a single class of particulate prey (Abrams 1990). To assess the importance of species-specific differences in functional responses, parameter estimates for the three species will be compared. This will involve estimating experimentally the parameters of the functional responses for a range of size and species combinations and testing for species effects against the statistical null hypothesis of no species difference.

Materials and Methods

We investigated feeding ability as a function of body size during ontogeny in three common Great Lakes fishes, alewife (*Alosa pseudoharengus*), yellow perch (*Perca flavescens*), and bloater (*Coregonus hoyi*). These species are from different families and hatch at substantially different sizes: alewife hatch at the smallest size, approximately 3.8 mm (Heinrich 1981); yellow perch are intermediate in size, hatching at about 5.5 mm (Scott and Crossman 1973; Cucin and Faber 1985); and bloater hatch at the largest size of the three species, approximately 9.8 mm (Rice et al. 1987). These three species span >80% of the size range of pelagic marine and freshwater fish at hatching. Although they differ substantially in morphology, they do not fully reflect the variety of morphologies found in fishes in general (Moser 1981). In Lake Michigan, larvae of the three species show considerable spatial and temporal overlap (Wells 1980; Auer 1982; Rice et al. 1987).

Experiments were conducted from May to September 1989 at the Center for Great Lakes Studies, Milwaukee, Wisconsin. All fish used in experiments were raised in the laboratory from eggs obtained from natural populations. Peak hatching of bloater occurred on May 5–6 with a mean total length (TL) of 9.67 ± 0.22 mm (mean $\pm$ SD). Peak hatching for the Green Bay strain of yellow perch occurred on May 12 and for the Lake Michigan strain on June 25. The average TL's at hatch were 5.32 ± 0.32 and 5.93 ± 0.44 mm (mean $\pm$ SD), respectively. Peak hatching of alewife occurred on August 3, with mean TL at hatch of 3.87 ± 0.54 mm (mean $\pm$ SD). After hatching, larvae of all three species were transferred to fiberglass tanks, fed on a mixture of *Artemia* supplemented with frozen commercial feed, and maintained on a 12-h light : 12-h dark cycle at 12–15°C until required for an experiment.

To describe the functional response of each size and species of fish, we exposed fish to a range of five different *Artemia* densities (nominal concentrations of 10–1000 nauplii/L). Although *Artemia* is not a natural prey, its use does allow feeding abilities to be investigated independent of differential prey effects. All functional response trials were conducted in 20-L aquaria maintained under gentle aeration at 15°C, a temperature typically experienced by all three species in the wild.

We used a volumetric technique to generate the prey densities required in the experiments. Prior to all experiments, we determined the relationship between the weight of *Artemia* added to an aquarium and the prey concentration produced. For each set of trials, the wet weight of *Artemia* required to generate the desired prey concentration, calculated as wet weight (grams) = 3.022×10^{-4} prey concentration (number per litre) + 0.0141, $r^2 = 0.997$, $n = 49$, was divided by the weight of a standard volume of naupliar suspension (grams per litre) to estimate the volume of suspension (litres) to be added to the aquaria.

We conducted two replicates of each fish size and species combination. The sizes and ages of fish used in the experiments are given in Table 1. To initiate an experiment, 10 similarly sized fish were added to each aquarium and starved for 24 h to standardize experimental conditions and to empty their guts. All fish used in trials were within 2 mm of the target length. Immediately prior to each set of trials, we siphoned the bottom of the aquaria to remove extraneous material and placed four rubber stoppers randomly on each tank bottom. The experimental rations were then added to the aquaria. Gentle aeration kept the *Artemia* suspended and well distributed in the tank.

Fish <20 mm were exposed to the nauplii for 30 min and those ≥ 20 mm for 15 min. These times were based on preliminary trials, as they balanced the need to allow fish to fill their guts at the higher prey concentrations, but did not allow them to deplete the resource significantly (maximum change in prey concentration $\approx 20\%$) or to defecate material. To assess prey densities at the end of the prescribed feeding period, we lowered four PVC tubes over the stoppers, thus sampling a volume of water and the *Artemia* it contained. The fish were then netted out of the aquaria and killed with MS222. In the laboratory, the nauplii in each fish stomach were removed and counted. The tube samples were poured into measuring cylinders, their volumes recorded, and sieved through 64- μ m Nitex. The nauplii retained on the sieve were preserved in 5% formalin for later enumeration and subsequently counted and converted to prey concentrations.

Functional response data were analyzed for goodness of fit to Holling's type II equation, defined by

$$(1) \quad N_{\text{eaten}} = \frac{a \cdot T_t \cdot P}{1 + a \cdot T_h \cdot P}$$

where a is the instantaneous rate of discovery or attack constant (attacks per second). This parameter is the product of the animal's rate of searching and the probability of finding a prey and combines both the encounter and attack components of predation (Holling 1959). The other parameters of the equation are T_t , the total time available for foraging (seconds), T_h , the handling time per prey (seconds), and P , the prey concentration (number per litre) (Holling 1959, 1966). Parameter estimates ($\pm$ SE) were obtained using Marquardt's method of nonlinear regression (Procedure NLIN; SAS Institute 1985). Data from the two trials were combined to produce more accurate estimates of a and T_h (Houck and Strauss 1985). Prey saturation values, N_{max} , were estimated from T_h as

$$(2) \quad N_{\text{max}} = \frac{T_t}{T_h}$$

Parameter estimates were log transformed to stabilize variance. Analysis of covariance (ANCOVA) was used to detect species differences between the size-dependent relationships for the individual parameters (Snedecor and Cochran 1980). We

TABLE 1. Parameter estimates for Holling's type II functional response equation ($\pm$ asymptotic SE) as obtained from nonlinear regression. Regressions are based on the mean gut content data from two trials ($n = 10$). Estimated parameters are instantaneous rate of discovery, a , and handling time, T_h . Prey saturation levels, $N_{\max}$, are calculated using Eq. 2.

Species	TL (mm)	Age (d)	Holling equation			
			a (s^{-1})	T_h (s)	R^2	$N_{\max}$ (no./900 s)
Alewife	10	10	2.85×10^{-5} (6.2×10^{-6})	58.12 (16.58)	0.98	15.5
	15	12	5.2×10^{-7} (7×10^{-10})	0 0	—	—
	20	36	0.0028 (0.003)	3.47 (1.34)	0.71	259.4
	30	37	0.0021 (9.7×10^{-4})	0.88 (0.36)	0.95	1023.9
	40	45	0.054 (0.023)	0.94 (0.084)	0.98	954.1
Bloater	10	8	2.4×10^{-4} (9×10^{-5})	118.41 (8.56)	0.98	7.6
	15	37	0.0039 (0.006)	39.15 (9.42)	0.91	23.0
	20	37	0.015 (0.0063)	3.19 (0.315)	0.96	281.9
	30	67	0.024 (0.0026)	1.39 (0.038)	0.99	646.6
	40	96	0.063 (0.017)	0.531 (0.038)	0.96	1694.9
Yellow perch	10	6	3.6×10^{-5} (2×10^{-5})	53.62 (24.94)	0.94	16.8
	15	31	0.0014 (0.001)	8.80 (1.56)	0.97	102.4
	20	34	0.0011 (2×10^{-4})	2.13 (0.30)	0.99	423.1
	30	65	0.0019 (7×10^{-4})	1.49 (0.43)	0.97	606.1
	40	94	0.014 (0.005)	0.61 (0.089)	0.98	1478.5

tested for differences in the slopes of the lines using an F test for the species $\times$ size interaction effect in a full model. We set $\alpha = 0.1$ in these tests to protect the power of the test (Cohen 1988). When we could not reject the assumption of equality of slope, the adjusted means for species were tested using an F test for species in the reduced model at $\alpha = 0.05$. In all cases the assumption of linearity of the parameter estimates with the covariate was checked and could not be rejected. When between-species differences could not be detected a general size-dependent relationship was reported.

We estimated the statistical power (Peterman 1990) of the tests to assess the strength of our inferences using relationships given by Cohen (1988). The power, $(1 - \beta)$, of the test of a null hypothesis is the probability that it will lead to rejection of a false null hypothesis.

Results

The functional responses of all combinations of species and sizes were described well by Holling's type II functional response equation (Fig. 1), except in the case of 15-mm alewife (Table 1). With this exception, the R^2 values were consistently high (mean = 0.95, range = 0.71–0.99). Fifteen millimetre alewife failed to feed at prey concentrations less than 200 prey/L. Consequently, nonlinear regression for this size and species

failed to converge on a set of positive values. These data were therefore excluded from the analysis. ANCOVA revealed no significant differences between the slopes of the regression relationships for the three species or between the adjusted means for the three species for either parameter of Holling's equation (Table 2). Although the power of the tests for the instantaneous rates of discovery is good, the power of the tests for the handling times is low. Generally, $(1 - \beta) \geq 0.8$ is considered acceptable. The calculated power of these tests suggests that we would fail to reject the null hypothesis of no difference between the slopes, if it were false, about 3 times out of 10.

Body size significantly influenced both the instantaneous rate of discovery or attack constant, a ($p < 0.008$), and the handling time, T_h ($p < 0.006$) (Table 2; Fig. 2). As ANCOVA did not detect differences among the three species, we developed a general size-dependent relationship for each parameter. Predicted instantaneous rates of discovery or attack constant increased with fish size in all three species (Fig. 2A). In contrast, predicted handling times decreased with fish size (Fig. 2B). The size-dependent relationships we developed for individual species explained between 82 and 95% of the variation in the log-transformed data. The general size-dependent relationships explained 71 and 91% of the variation in the data, respectively.

We converted T_h values to their $N_{\max}$ equivalents to evaluate how the patterns in prey saturation values varied among the

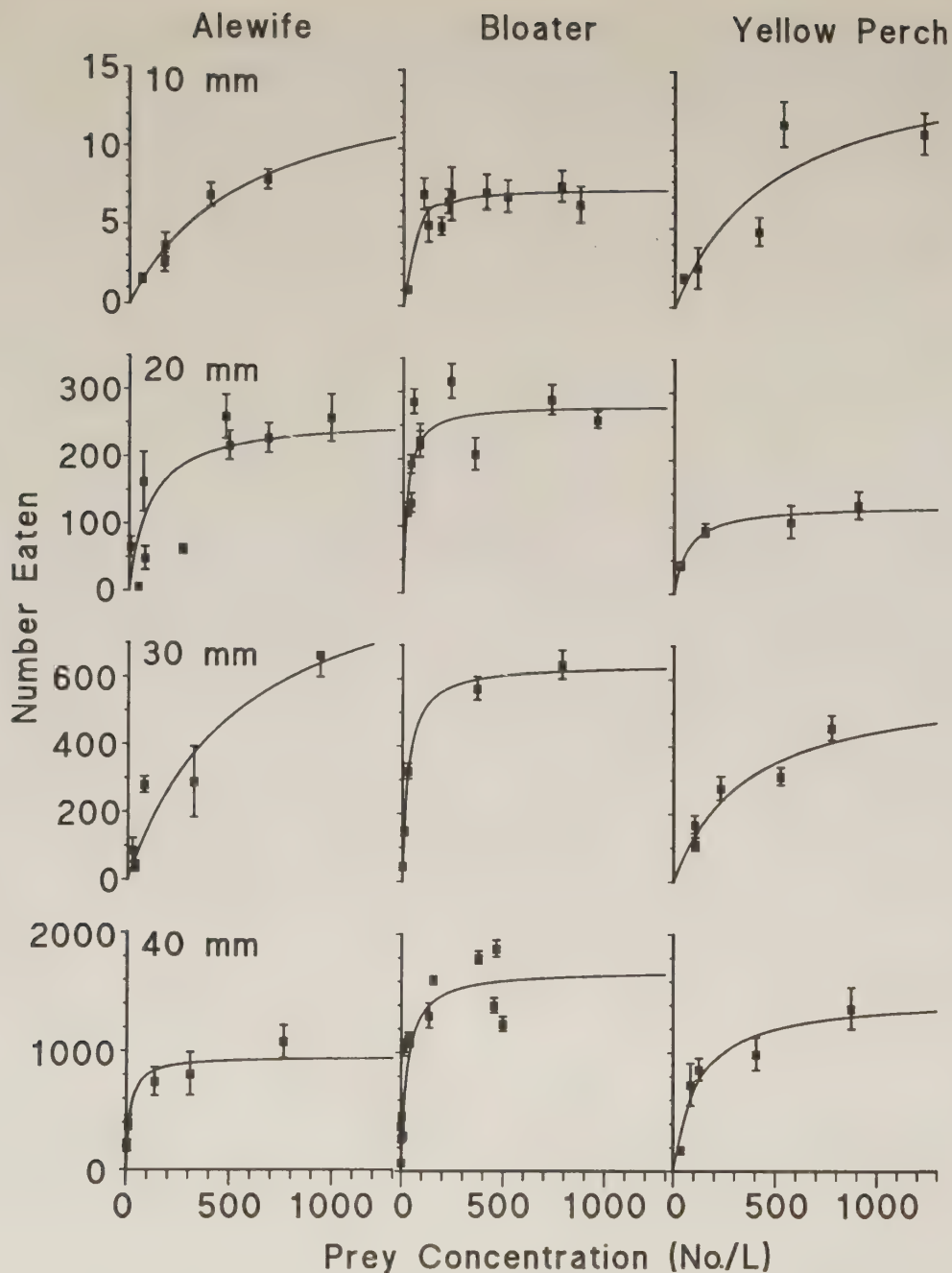


FIG. 1. Nonlinear regression estimates of functional response relationships based on Holling's type II equation (Eq. 1). Individual data are mean gut contents ($\pm$ SE) of 10 like-sized fish from a single tank trial standardized to a 15-min exposure to a single concentration of *Artemia* prey.

species. $N_{\max}$ is a simple transformation of T_h . Therefore, ANCOVA results for the two variables are identical. We developed a general size-based relationship for prey saturation values. Species-specific prey saturation values regressed against body size explained 91–95% of the variation present (Fig. 3). The combined data explained 91% of the variation in the data (Fig. 3).

Discussion

If one begins to examine the foraging abilities of larvae of an unstudied species, what insights can be gained from a review of the literature of the ontogeny of feeding in other species? One view, common in the literature, is that species are so dif-

ferent that meaningful generalizations are improbable (Lasker 1987). This view, if fully accepted, leads to the requirement that each species for which no information is available be studied in detail. More recently, Miller et al. (1988) have suggested that larval size may be a character on which to base interspecific comparisons. If this view can be supported, then general relationships derived from a review of well-studied species could be used to develop expectations for relatively unstudied species.

In this study, we attempted to verify the validity of the latter view by assessing the foraging ability of three species of fishes. Despite species differences in the measures of foraging we considered, our data support the notion that body size accounts for a sizeable portion of the overall variation and that species-specific differences are most effectively resolved after first

TABLE 2. Summary of ANCOVA results and tests of power, $(1 - \beta)$, for the parameters of Holling's type II functional response equation. All analyses were conducted on log-transformed values of both the independent and dependent variables.

Instantaneous rate of discovery					
Source	df	SS	F	p	(1 - β)
Equality of slopes					
Species	2	2.73	1.3	0.33	0.76
Size	1	12.06	11.07	0.008	0.94
Sp. $\times$ size	2	1.79	0.8	0.47	0.76
Error	9	9.81			
Equality of adjusted means					
Species	2	5.87	2.78	0.11	0.76
Size	1	12.06	11.4	0.006	0.94
Error	11	11.61			
Handling time					
Source	df	SS	F	p	(1 - β)
Equality of slopes					
Species	2	0.1737	1.5	0.27	0.64
Size	1	7.8289	139.3	0.0001	0.99
Sp. $\times$ size	2	0.1347	1.2	0.35	0.67
Error	8	0.452			
Equality of adjusted means					
Species	2	0.1272	1.09	0.37	0.64
Size	1	7.8941	135.10	0.0001	0.99
Error	10	0.581			

accounting for differences associated with larval size. It is important to emphasize that we do not conclude from our inability to detect species-specific differences among these three species that they do not exist or that they may not be important in other cases. Considering body size alone clearly does not explain all of the variation in feeding abilities among all species. Yet, it does represent a good first approximation.

Our inability to detect species-specific differences may result from two factors: statistical power and the species chosen for our experiments. Some of the tests we employed had lower power than the 0.8 considered acceptable (Cohen 1988), particularly the tests for handling times. Thus, we cannot deny the possibility of having failed to reject a false null hypothesis.

We may also have detected a species effect if we had chosen species that differed more in morphology or differed less in size. The three species were chosen because of their differences in hatching size, yet they do not fully represent the range of morphologies exhibited by larvae of different species (Moser 1981). A different set of species may have indeed yielded significant species effects. We acknowledge that species effects do exist, but suggest that in many cases they may not be so large as to deny the possibility of useful generalizations.

Further, the experiments were conducted using *Artemia* nauplii. This prey is known to be particularly vulnerable to fish predation, and thus its use may have obscured differences in ability or behavior that would have been apparent if a natural prey had been used. However, the use of a natural prey assemblage would have greatly complicated the interpretation of the results. It would have been extremely difficult to separate

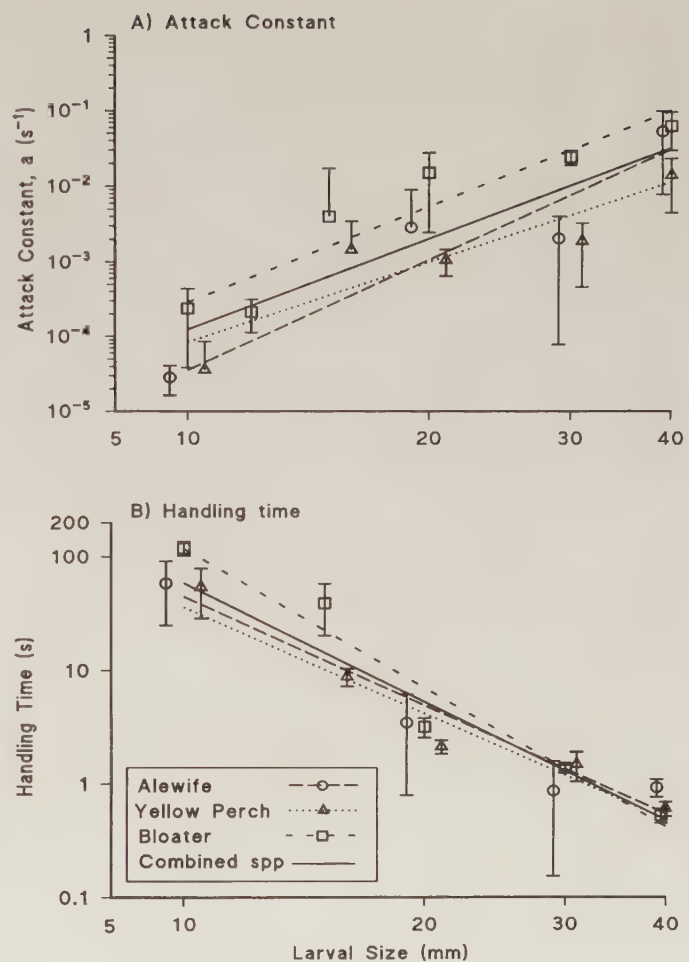


FIG. 2. Relationship between parameter estimates for Holling's type II equation and fish size. Data for alewife and yellow perch have been plotted at slightly smaller and slightly larger sizes, respectively, to improve the visual presentation. (A) Attack constants or instantaneous rate of discovery, a (± 2 SE). Individual species regressions: alewife, $\log(a) = 6.14 \cdot \log(\ell) - 10.77$, $r^2 = 0.83$, $n = 5$, $p < 0.05$; bloater, $\log(a) = 4.24 \cdot \log(\ell) - 7.78$, $r^2 = 0.88$, $n = 6$, $p < 0.05$; yellow perch, $\log(a) = 3.54 \cdot \log(\ell) - 7.62$, $r^2 = 0.82$, $n = 5$, $p < 0.05$. Combined taxa: $\log(a) = 4.43 \cdot \log(\ell) - 8.39$, $r^2 = 0.73$, $n = 16$, $p < 0.05$. (B) Handling time, T_h (± 2 SE). Individual species regressions: alewife, $\log(T_h) = 4.82 - 3.16 \cdot \log(\ell)$, $r^2 = 0.94$, $n = 4$, $p < 0.05$; bloater, $\log(T_h) = 6.22 - 4.06 \cdot \log(\ell)$, $r^2 = 0.95$, $n = 5$, $p < 0.05$; yellow perch, $\log(T_h) = 4.65 - 3.10 \cdot \log(\ell)$, $r^2 = 0.94$, $n = 5$, $p < 0.05$. Combined taxa: $\log(T_h) = 5.22 - 3.46 \cdot \log(\ell)$, $r^2 = 0.91$, $n = 14$, $p < 0.05$.

effects on feeding ability that resulted from differences in prey behavior, or prey distributions among trials, from those relating specifically to differences in the ability of the fishes themselves. As *Artemia* are not the natural prey of any of the species tested, our results may not be readily transferable to the field (MacKenzie et al. 1990).

Our finding that size-dependent patterns are important in the ontogeny of foraging ability in fishes is consistent with the results of other recent studies in this area. MacKenzie et al. (1990) recently reviewed the influence of body size, temperature, food density, and experimental protocol on the ingestion rates of larvae of 11 fish species. Their results, which are based on a multiple regression analysis of previously published data, suggest that body size is the single best predictor of ingestion rate, explaining nearly 60% of the variation in the data. When combined with data on the temperature at which experiments

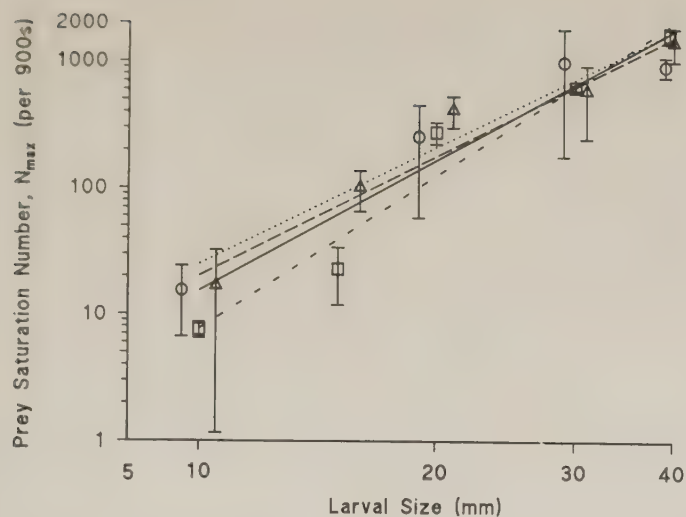


FIG. 3. Prey saturation values as a function of larval size (± 2 SE). Species data are as indicated in Fig. 2. Data for alewife and yellow perch have been plotted at slightly smaller and slightly larger sizes, respectively, to improve the visual presentation. The solid line indicates the general size-based function. Individual species values: alewife, $\log N_{\max} = 3.17 \cdot \log(\ell) - 1.86$, $r^2 = 0.94$, $n = 4$, $p < 0.05$; bloater, $\log N_{\max} = 4.05 \cdot \log(\ell) - 3.17$, $r^2 = 0.95$, $n = 5$, $p < 0.05$; yellow perch, $\log N_{\max} = 3.09 \cdot \log(\ell) - 1.69$, $r^2 = 0.94$, $n = 5$, $p < 0.05$. Combined taxa: $\log N_{\max} = 3.45 \cdot \log(\ell) - 2.26$, $r^2 = 0.91$, $n = 14$, $p < 0.05$.

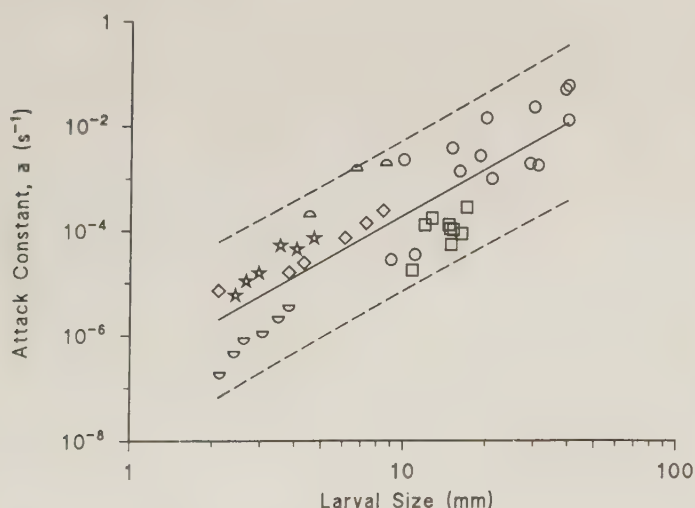


FIG. 4. Relationship between published attack constants or instantaneous rates of discovery and fish size for several species. The data were derived from the sources indicated below and were standardized to litres per second. Regression relationship for the combined data: $\log(a) = 2.94 \log(\ell) - 6.65$, $n = 48$, $r^2 = 0.73$, $p < 0.001$. Broken lines are 95% confidence intervals for single observations. Symbols: $\circ$, this study (sizes for alewife and yellow perch have been altered to improve clarity); $\square$, Atlantic herring (Werner and Blaxter 1981); $\diamond$, lined sole (Houde and Schekter 1980); $\star$, sea bream (Houde and Schekter 1980); $\circ$, winter flounder (Laurence 1975, 1977).

were conducted, these two variables explained over 80% of the variation in ingestion rates.

The results of functional response studies on other species also lend some support to our findings. We found a strong relationship between the attack constants or instantaneous rate of discovery, a , and size during ontogeny. Houde and Schekter (1980) observed that the instantaneous rate of discovery

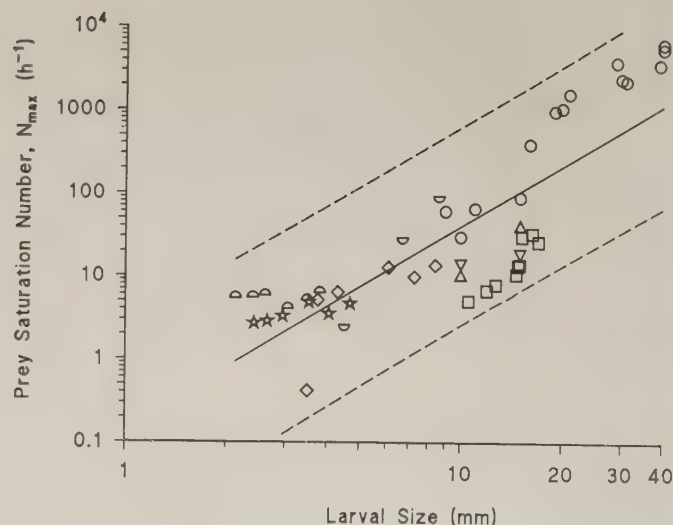


FIG. 5. Relationship between published saturation prey numbers and fish size for several species. Data have been standardized to numbers per hour. Regression relationship for the combined data: $\log N_{\max} = 2.45 \cdot \log(\ell) - 0.84$, $n = 48$, $r^2 = 0.71$, $p < 0.001$. Broken lines are 95% confidence intervals for single observations. Data sources are as detailed in Fig. 4, with the addition of the following: $\triangle$, Atlantic herring (Blaxter 1962); ∇ , Atlantic herring (Rosenthal and Hempel 1980).

increased markedly during early development in bay anchovy (*Anchoa mitchelli*), sea bream (*Archosargus rhomboidalis*), and lined sole (*Achirus lineatus*). But both Laurence (1975, 1977) and Werner and Blaxter (1981) found no evidence of similar trends in instantaneous rates of discovery in winter flounder (*Pseudopleuronectes americanus*) and Atlantic herring (*Clupea harengus*), respectively. However, in these studies the size ranges investigated were very small, 4.5–8.6 and 10–17 mm, respectively. Consequently, it may be difficult to detect a size-dependent relationship in these data. The exact nature of the size-dependent relationship for attack constants or instantaneous rates of discovery remains equivocal and deserves further study. However, there does appear to be a common pattern in the available data (Fig. 4). The degree to which the substantial variation in the data now available reflects real species-specific differences as opposed to consequences of methodological differences in estimating instantaneous rates of discovery remains to be assessed.

We found that saturation prey levels increased with body size in all species studied. Blaxter (1962), in a study of the functional response of Atlantic herring larvae, found that the saturation prey value increased from 10 to 40 *Artemia* nauplii as larvae grew from 10 to 15 mm standard length. Rosenthal and Hempel (1970) reported saturation prey values for 10- and 15-mm Atlantic herring feeding on *Artemia* to be 15–20 nauplii. Houde and Schekter (1980), who investigated the functional response of bay anchovy, sea bream, and lined sole, observed a 16–229% increase in the saturation prey value over a range of fish dry weights from 10 to 200 μg . Laurence (1975, 1977) and Werner and Blaxter (1981) found similar patterns in the prey saturation values of larval winter flounder and Atlantic herring. The generality of these findings is illustrated in Fig. 5, where we have combined the results of all studies, noted above, including our own. However, although size indeed seems to dominate the relationship overall, it should be noted that the size-dependent relationships within species may be different. But the potential deviation of individual taxa can only be rec-

ognized when the expected relationship is known. Similar patterns are found in many other allometric relationships (Peters 1983).

There have been relatively few studies of the size dependence of $N_{\max}$ in fishes. To permit comparisons among the studies, we expressed $N_{\max}$ as an exponential function of size. Stepien (1976) investigated the age dependence of $N_{\max}$ in sea bream feeding on prey at 1000/L at 23, 26, and 29°C. He reported the age dependence as a general exponential form. We converted his data to the general form using the length–age relationships he provided to yield exponent values of 2.96, 3.46, and 2.01, respectively, for the three different temperatures. The corresponding intercept values are 0.47, 0.53, and 3.17. Houde and Schekter (1980) reported $N_{\max}$ values as a function of age and weight. We expressed these data in the general form using length–age relationships in Houde and Schekter (1981). The equations for the three species are as follows: bay anchovy, $N_{\max} = 0.073 \cdot \ell^{2.72}$; sea bream, $N_{\max} = 0.079 \cdot \ell^{3.20}$; and lined sole, $N_{\max} = 0.37 \cdot \ell^{2.57}$. The relationships for the species we studied are as follows: alewife, $N_{\max} = 0.014 \cdot \ell^{3.16}$; bloater, $N_{\max} = 0.006 \cdot \ell^{4.05}$; yellow perch, $N_{\max} = 0.020 \cdot \ell^{3.10}$. Although the exponent values reported compare favorably, this is not true of the intercept values. The reasons for this wide discrepancy are unclear. The intercept values dominate the $N_{\max}$ function when fish are small, and thus these differences may have large potential effects on growth rates of larval and juvenile fishes. Their resolution will require a fuller investigation of the nature of size dependency and prey saturation values in fishes.

To conclude, we have documented clear size dependencies in the functional responses of larval and juvenile fish during development. Alewife, yellow perch, and bloater dramatically improved their foraging abilities as they developed, in accordance with several other species studied recently. Species-specific differences, while present, were not statistically different, possibly as the range of larval types was too restrictive and the range of sizes too broad. This constraint notwithstanding, we feel that the evidence presented here and elsewhere is sufficient to support the hypothesis that the importance previously accorded to species-specific differences has been overemphasized. This results, we believe, from a failure to quantitatively standardize components of foraging ability for larval size or temperature prior to making interspecific comparisons. We do not suggest that species are unimportant and that fish size is the sole determinant of foraging ability during the fish early life history. We do conclude, however, that body size can provide first-order predictions of the probable foraging abilities of young fishes across a broad range of taxa. Such an approach would allow investigators to identify, and study, species that deviate from the general expectation. This approach should enhance our ability to understand more fully the dynamics of foraging in larval fishes and its relevance to growth and survival.

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Foraging and Prey Search Behaviour of Golden Shiner (*Notemigonus crysoleucas*) Larvae

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Browman, H. I., and W. J. O'Brien. 1992. Foraging and prey search behaviour of golden shiner (*Notemigonus crysoleucas*) larvae. *Can. J. Fish. Aquat. Sci.* 49: 813–819.

The juveniles of several species of freshwater fish search for zooplankton prey using a strategy intermediate between cruise and ambush: "saltatory search" (SS) or "pause-travel" search. Unlike ambush or cruise search, saltatory search involves scanning for prey throughout the search space and only during the brief stationary periods that punctuate repositioning movements. If no prey are located, these fish swim a short distance, stop, and scan again. In this paper, we describe the ontogeny of prey search in a cyprinid, the golden shiner (*Notemigonus crysoleucas*), a species whose search pattern has not been examined. Swimming and pursuit speeds and prey location distances increased with fish size. Golden shiner larvae searched for prey throughout the search space and only during the pauses that punctuated swimming movements. Only 1–10% of all of the stationary pauses that punctuated swimming movements were followed by attacks on prey. We conclude that golden shiner larvae employ a saltatory-search strategy similar to that described in other zooplanktivorous fish and their larvae.

Les juvéniles de plusieurs espèces de poissons d'eau douce chassent leurs proies zooplanctoniques en utilisant une stratégie intermédiaire entre le déplacement continu et l'affût : la stratégie «saltatoire» (SS) ou «d'arrêt et déplacement». À la différence de la recherche de proies par déplacement continu ou à l'affût, la stratégie saltatoire consiste à localiser les proies dans tout l'espace de recherche seulement durant les courtes périodes stationnaires entre les déplacements. Si aucune proie n'est localisée, ces poissons nagent sur une courte distance, s'arrêtent, puis tentent à nouveau de localiser des proies. Dans le présent article, nous décrivons l'ontogénie du comportement de recherche de proies d'un cyprinidé, la chatte de l'est (*Notemigonus crysoleucas*), comportement qui n'a jamais été étudié chez cette espèce. La vitesse à laquelle elle nage et poursuit ses proies de même que la distance à laquelle elle peut localiser ses proies s'accroissent en raison directe de sa taille. Les larves de la chatte de l'est recherchent leurs proies dans tout l'espace de recherche et seulement durant les pauses entre leurs déplacements. Seulement 1 à 10 % des pauses stationnaires ont été suivies d'une tentative de capture de proie. Les larves de la chatte de l'est ont recouru à une stratégie saltatoire semblable à celle déjà observée chez d'autres poissons zooplanctivores et leurs larves.

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Zooplanktivorous fish are often important predators in aquatic ecosystems and are known to have pronounced effects on the population dynamics of their prey (Lazzaro 1987). To elucidate their role as predators, the feeding modes, selectivities, and search patterns of juvenile and adult planktivores have all been examined in some detail (O'Brien 1979; O'Brien et al. 1986, 1989; Lazzaro 1987; Wetterer 1989; O'Brien et al. 1990).

Virtually all fish are planktivorous at some time during ontogeny, and because of the importance of early life history stage dynamics to fisheries biologists (e.g. Sinclair 1988), the foraging behaviour of fish larvae has been studied extensively (Hunter 1980; Brown and Colgan 1984, 1985; Blaxter 1986; Noakes and Godin 1988). Until recently, however, the ontogeny of prey search strategies in fish larvae, and how these relate to those of juveniles and adults, has received only limited attention (Browman and O'Brien 1992).

Animals, such as zooplanktivorous fish, that forage for discrete, isolated resources are generally characterized as either "ambush" (sit-and-wait) or "cruise" (active) searchers

(McLaughlin 1989; Tye 1989; Bell 1990; O'Brien et al. 1990). Ambush foragers have been characterized as stationary searchers, scanning for prey at the periphery of their strike range. They typically wait for prey to cross the outer boundary of the search space before attacking. Cruise foragers have been characterized as moving more or less continuously, scanning for prey near the outer boundary of their search space. An intermediate search strategy, termed "saltatory search" (SS) by O'Brien et al. (1989, 1990) and "pause-travel" search by Tye (1989), has recently been described in the juveniles of several species of zooplanktivorous fish and in several other taxonomic groups (e.g. Janssen 1982; Ehlinger and Wilson 1988; Ehlinger 1989a; Tye 1989; Ehlinger 1990; reviewed by Bell 1990; O'Brien et al. 1990). Saltatory-searching animals scan for prey throughout the search space, but only during the brief stationary periods that punctuate swimming movements. If prey are not located, the animal moves a short distance, stops, and scans again (O'Brien et al. 1986, 1989, 1990; Evans and O'Brien 1988; Tye 1989).

In a previous study, we examined the ontogeny of prey search in a centrarchid, the white crappie (*Pomoxis annularis*), and found that the larvae of this species employ a search strategy similar to that observed in juveniles (Browman and O'Brien 1992). In that study, we discussed how characterizations of a

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fish's search pattern could affect the calculation and interpretation of such commonly estimated parameters as prey encounter and feeding rate. In this paper, we examine the generality of the saltatory-search pattern and report the results of experiments in which the ontogeny of foraging and prey search behaviour in the golden shiner (*Notemigonus crysoleucas*) was examined.

Study Species

Golden shiner are widely distributed in North America, commonly inhabiting weedy lakes, ponds, and streams (Scott and Crossman 1973). The species is often one of the most abundant of the ichthyofauna and is a significant consumer of zooplankton biomass (Hall and Ehlinger 1989) as well as an important forage fish in the diet of several gamefish species (Scott and Crossman 1973; Keast 1985; Johannes et al. 1989). Adults (50–70 mm total length (TL)) are usually found in midwater or near the surface in small shoals (Hall et al. 1979), while larvae in mixed-cohort shoals are found in the nearshore littoral zone (Hatch 1988). Eleutheroembryos are approximately 3 mm in length, and fish of 5.0–14.0 mm TL are considered larvae. Transition to the juvenile period occurs at approximately 18 mm TL. The species' development is summarized in Auer (1982, p. 223–228). To our knowledge, no detailed observations on the foraging behaviour of golden shiner larvae have been published.

Materials and Methods

General Procedures

Fertilized golden shiner eggs, deposited on artificial brush nests, were obtained in July 1988 from the Kaw Valley Fish Farm in Douglas County, Kansas, USA. The nests were incubated in aquaria at 26°C on a 14 h light : 10 h dark cycle. Eleutheroembryos were 3.9 ± 0.23 mm TL ($n = 6$) and prey items were first observed in the gut of fish 4.77 ± 0.45 mm in length ($n = 6$) on day 3 post-hatch (DPH).

Larvae were fed on fresh zooplankton, collected from local reservoirs, until they reached a size of 15 mm. Larvae >15 mm were fed a mixture of zooplankton and *Artemia* nauplii (Argent Laboratories' "Argentemia Silver"). Total prey abundance was $500 \cdot L^{-1}$.

Observations began 4 d after food was first observed in the gut, after the yolk sac had been completely absorbed. The mean total lengths ($\pm$ SD, $n = 5$) of fish observed in the experiments were 6.22 ± 0.24 , 8.44 ± 0.22 , and 21.4 ± 0.9 mm. According to the criteria summarized in Auer (1982, p. 223–228), the fish in the 21.4-mm size class were recently transformed juveniles. When larvae reached 8 mm TL, their numbers had been greatly reduced by mortality. As a result, surviving fish were used in several experiments as they grew.

In all experiments, five fish were placed in a $30 \times 30 \times 30$ cm all-glass observation tank at least 12 h prior to an experiment. The tank was filled with 12 L of water and was devoid of prey items. Its sides were covered with black plastic. Light intensity (diffused sunlight) at the water surface was 100 lx (± 10) and water temperature was 26°C.

At the beginning of an experiment, food items (zooplankton that passed through a 64- μm mesh) were introduced to the observation aquarium at an abundance of $100 \text{ prey} \cdot L^{-1}$. No clumping of prey was apparent. Prey consisted of copepod nauplii or early copepodites and small cladocerans upon which golden shiner larvae feed in the wild (Keast 1980; Hatch 1988).

The abundance of prey items introduced in the experiments was determined by calculating the mean number of items in three 1-mL subsamples of the source populations and introducing the volume required to yield $100 \text{ items} \cdot L^{-1}$ in the 12-L observation tank. In each experiment, foraging behaviour was videotaped until the five fish had attempted 10–15 attacks on prey. All experiments were conducted between 11:00 and 13:00 and lasted 30 min.

Observations and Analysis of Foraging and Search Behaviour

Silhouette (shadow) video photography was used to record the foraging and prey search behaviour of golden shiner larvae (Arnold and Nutall-Smith 1974). Shadow images were focused on the lens (Nikon 35 mm f/2.0) of Panasonic WV-140 black and white (b&w) videocameras, recorded with Panasonic NV-8420 and General Electric 1CVD5025X videotape recorders, and monitored on b&w television screens.

A small, under-run microscope lamp (12 V, 100 W, filament area 4×2 mm) was used as a point source of light and placed at the first focus of an optical-quality biconvex collimating lens (18 cm in diameter, 333-mm focal length, Edmund Scientific). The lens was oriented for imaging in the vertical plane (from above). To estimate the extent of vertical movement during foraging, a second lens, oriented for synchronous imaging in the horizontal plane (from the side), was used in some experiments. From the point sources of light, these lenses "projected" a collimated beam through the observation tank. The use of a collimated beam prevented perspective distortion; clear, sharp shadows of any organism (even transparent ones) in the beam's path were produced and recorded by the videocameras. The system's resolution was approximately 0.2 mm.

The videotapes were analyzed frame by frame on a video-monitor using a Panasonic AG-1950 videotape machine. All time intervals were measured in increments of 0.033 s. For each experiment, a videotaped ruler established conversions from monitor units to millimetres. Sequences in which movement in the vertical plane exceeded 5° from horizontal were not analyzed (approximately 5% of all sequences).

Search behaviour was analyzed by assigning the activities of the fish to one of the components of their predation cycle: pause, move (swim), or pursuit (Fig. 1a). The fish's swimming movements are abbreviated by pauses, during which the fish is stationary. Pauses can be followed by moves or by pursuits. A move is operationally defined as a swimming movement that is not followed by an attack on prey. A pursuit is a swimming movement that precedes an attack on prey. Pauses prior to moves are termed unsuccessful search times (USST). Pauses prior to pursuits are termed successful search times (SST). The distance from the point at which the fish first reacted to a prey item and the position of the prey itself is the pursuit distance. In this analysis, pursuit distance is interpreted as equivalent to prey location distance.

Move and pursuit distances and angles were marked on the monitor with a grease pencil and measured (Fig. 1b). The longitudinal body axis of the fish was defined as the central axis of the forward-directed visual field (i.e. 0° from forward-directed). Thus, pursuit angle is defined as that angle between the central axis of the fish prior to pursuit and the line connecting the fish's rostrum to the position of the prey (determined as the point at which the fish attacked the prey item, or directly when the prey item could be seen on the screen) (Fig. 1b). Move turn angles were measured as the angle between the fish's body axis at one position and the position of its rostrum at the next (Fig. 1b). The durations of these events

a) Search cycle of golden shiner larvae

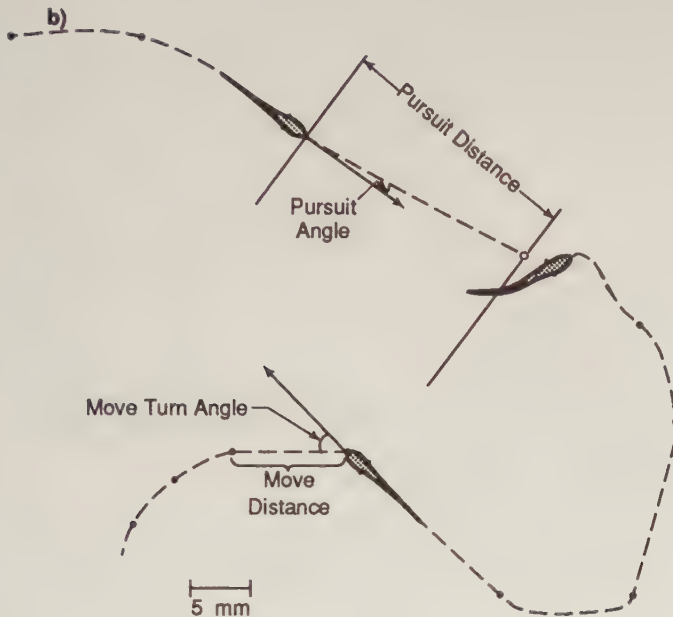
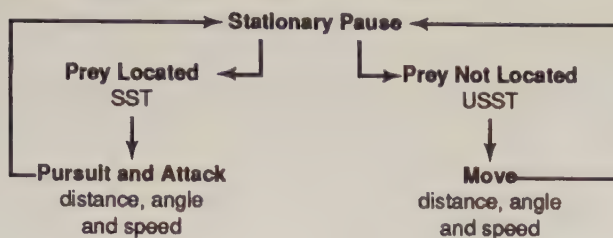


FIG. 1. (a) Flow chart of the predation cycle for golden shiner larvae. (b) A typical search path and attack sequence for golden shiner larvae, sketched directly off of the television screen, illustrating the measurements drawn from it. The solid dots along the broken line represent stationary pauses. The open dot represents a prey item.

and of stationary pauses between them were measured. Move and pursuit speeds were calculated from these measurements.

A multivariate analysis of variance, with a repeated measures design (Statistical package for the social sciences, routine MANOVA), was used to test for the overall effect of fish size on all of the components of the predation cycle. The univariate *F*-tests calculated from the MANOVA were used to evaluate whether fish size had an effect on any isolated component of the predation cycle.

Results

Swimming in golden shiner larvae was punctuated by frequent stops (every 0.17–1.67 s) and reorienting turns (Fig. 1b). Even the smallest individuals observed were relatively strong swimmers, moving through the water without the prolonged periods of rest exhibited by some other fish larvae (e.g. Rosenthal 1969; Hunter 1972; Drost and van den Boogaart 1986; Drost 1987). In any given experiment, approximately 1–10% of stationary pauses were followed by attacks on prey. The entire predation sequence, from prey location through attack, took an average of 1.2 s and did not vary significantly with fish size.

Fish size had a significant overall effect on the components of the golden shiner larvae predation cycle (MANOVA, Wilks lambda, $F = 5.328$, $P < 0.001$, $df = 20$). Swimming

TABLE 1. Measures of each component of the search cycle for three size classes of golden shiner larvae. Size class 1 = 6.22 ± 0.24 mm TL; size class 2 = 8.44 ± 0.22 mm TL; size class 3 = 21.4 ± 0.90 mm TL.

Variable	Fish size class	Mean	SE	n
Unsuccessful search time(s)	1	0.27	0.03	15
	2	0.06	0.01	15
	3	0.31	0.07	15
Move distance (mm)	1	0.34	0.05	30
	2	0.72	0.04	30
	3	0.76	0.11	30
Move time(s)	1	0.21	0.02	15
	2	0.44	0.01	15
	3	0.34	0.08	15
Move speed ($\text{mm} \cdot \text{s}^{-1}$)	1	13.5	2.2	15
	2	18.7	0.8	15
	3	27.5	4.6	15
Move angle (degrees)	1	49.18	4.99	30
	2	34.00	4.85	30
	3	76.57	5.84	30
Successful search time(s)	1	0.13	0.03	10
	2	0.18	0.05	12
	3	0.34	0.09	15
Pursuit distance (mm)	1	0.44	0.06	10
	2	0.63	0.07	12
	3	1.11	0.18	15
Pursuit time(s)	1	1.04	0.12	10
	2	1.11	0.10	12
	3	0.71	0.10	15
Pursuit speed ($\text{mm} \cdot \text{s}^{-1}$)	1	4.6	0.7	10
	2	5.9	0.6	12
	3	15.9	1.3	15
Pursuit angle (degrees)	1	40.70	6.87	10
	2	40.42	6.84	12
	3	40.13	6.74	15

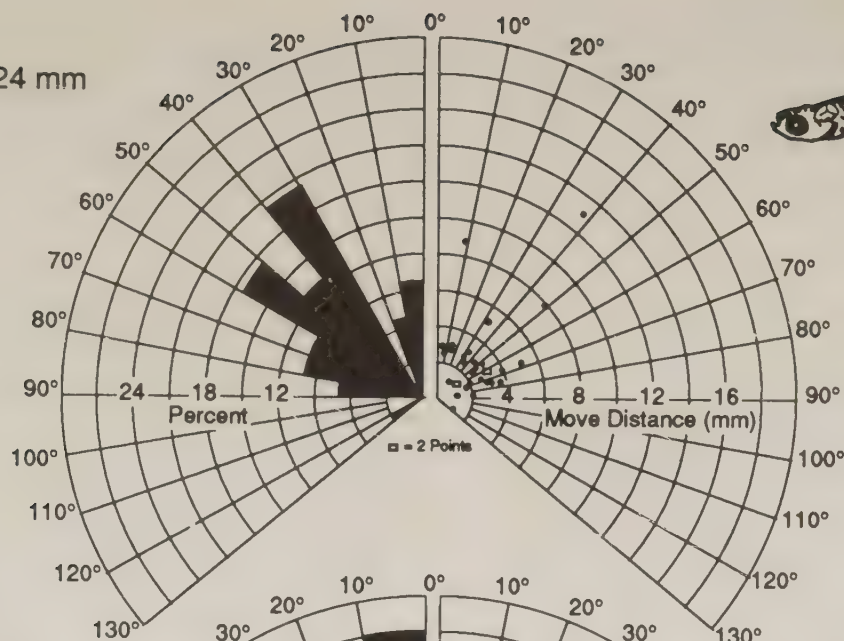
TABLE 2. Summary of the univariate *F*-tests (2,34 df), produced from the multivariate analysis of variance, testing for the effect of fish size on each component of the golden shiner larvae predation cycle.

Variable	<i>F</i> -value	Significance of <i>F</i> -value
Unsuccessful search time	7.367	0.002
Move distance	4.742	0.015
Move time	2.621	0.087
Move speed	6.847	0.003
Move angle	16.591	<0.001
Successful search time	2.267	0.119
Pursuit distance	6.554	0.004
Pursuit time	4.590	0.017
Pursuit speed	37.996	<0.001
Pursuit angle	0.0017	0.998

(move) speed increased with fish size (Tables 1 and 2). Mean move distance was greater for the two larger size classes of larvae than for the smallest size class (Tables 1 and 2; Fig. 2). The duration of move pauses (USST) was shorter in the second larval size class than in the other two (Tables 1 and 2). Mean move angle was greatest for the largest size class (Tables 1 and 2) and the distribution of move angles appeared to skew towards larger angles as the fish got larger (Fig. 2).

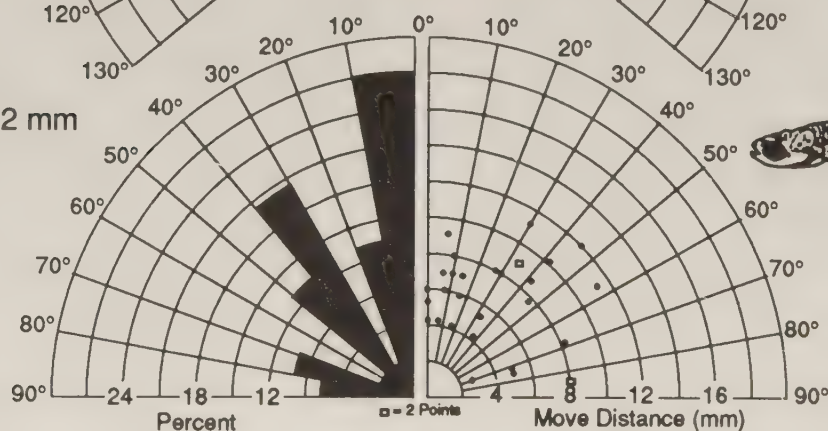
$\bar{X}TL = 6.22 \pm 0.24 \text{ mm}$

$n = 30$



$\bar{X}TL = 8.44 \pm 0.22 \text{ mm}$

$n = 30$



$\bar{X}TL = 21.4 \pm 0.9 \text{ mm}$

$n = 30$

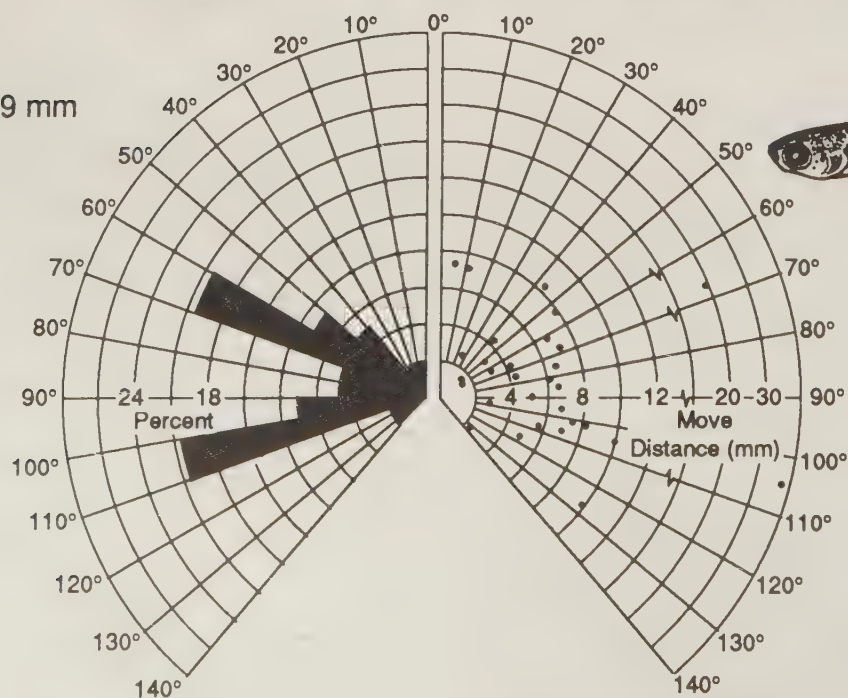
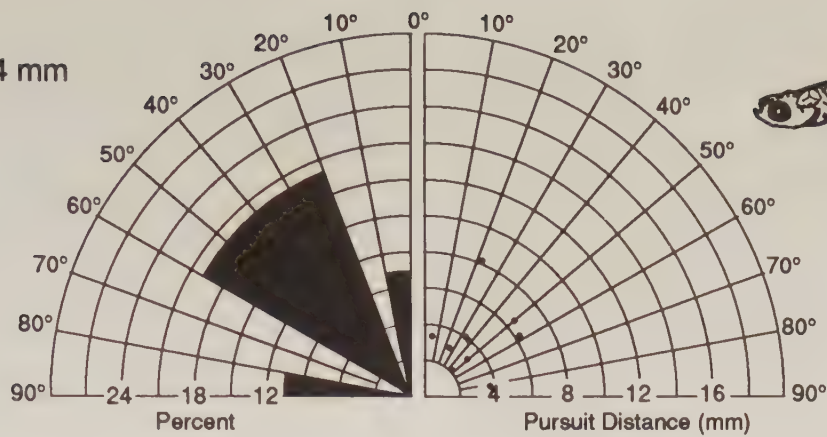


FIG. 2. Move distances and angles (both left- and right-directed) for each of three size classes of golden shiner larvae. Distances are plotted on the right and the percent frequency of locations in each 10° increment of the horizontal visual field is plotted on the left. The points are plotted as if the fish's eyes were positioned in the centre of the polar projection.

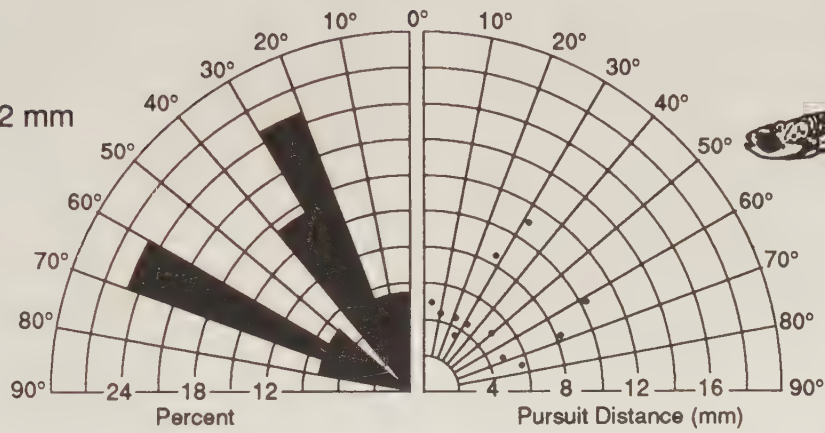
$\bar{X}TL = 6.22 \pm 0.24 \text{ mm}$

$n = 10$



$\bar{X}TL = 8.44 \pm 0.22 \text{ mm}$

$n = 12$



$\bar{X}TL = 21.4 \pm 0.9 \text{ mm}$

$n = 15$

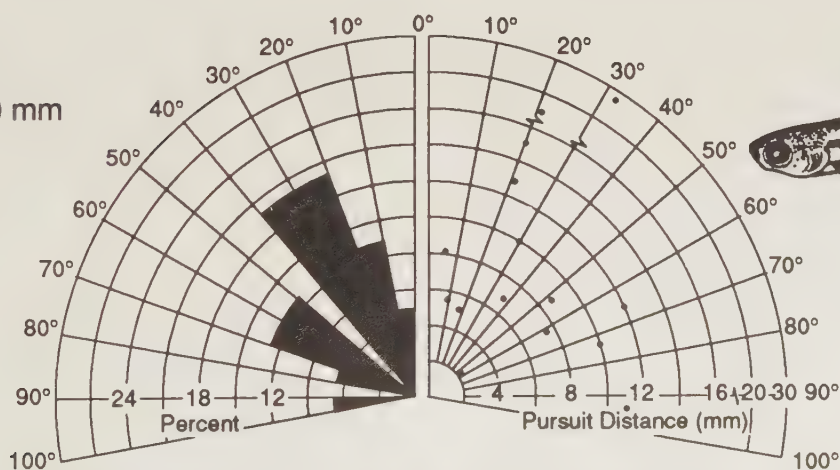


FIG. 3. Pursuit distances and angles (both left- and right-directed) for each of three size classes of golden shiner larvae. Distances are plotted on the right and the percent frequency of locations in each 10° increment of the horizontal visual field is plotted on the left. The points are plotted as if the fish's eyes were positioned in the centre of the polar projection.

Golden shiner larvae typically turned toward a located prey item and approached it by means of rapid tail beats. As they came very near to the prey, they braked using the pectoral fins and sucked the prey into their mouths by rapid expansion of the buccal cavity. No S-posture-type attacks were observed. Pursuit distances increased significantly with fish size (Tables 1 and 2; Fig. 3). The mean values for the duration of location pauses (SST) increased with fish size (Table 1), but these differences were not statistically discernible (Table 2). There was

no discernible relationship between mean pursuit angle and fish size (Tables 1 and 2), although the range of pursuit angles appears to have increased (Fig. 3). Pursuit speeds increased with fish size (Tables 1 and 2).

Discussion

Foraging Behaviour of Golden Shiner Larvae

Although the larvae of several fish species employ an S-posture in attacks on prey (e.g. Hunter 1972; Drost 1987;

Browman and O'Brien 1992), golden shiner larvae did not. Rather, shiner larvae feeding strikes were incorporated into more typical swimming movements, as is true for juvenile zooplanktivorous fish.

Recently, facultative switching of foraging modes has been reported in juvenile golden shiner (Ehlinger 1989b). Juvenile shiner are visually guided particulate-feeders when feeding on large prey and switch to a pump filter-feeding mode, during which their movements are apparently not visually guided, when feeding on high densities of small zooplankton. Further, when particulate-feeding, juvenile shiner were reported to wait 5–10 captures before pausing to swallow the accumulated bolus of prey (Ehlinger 1989b). We did not observe this behaviour, nor pump filter-feeding, perhaps because the prey that we used in our experiments were large relative to the shiner larvae themselves.

Search Strategy of Golden Shiner Larvae

The size-related changes in the components of the golden shiner predation cycle that we observed are similar to those reported for herring, anchovy, and other centrarchids and cyprinids (Hunter 1980; Brown and Colgan 1985; Blaxter 1986; Noakes and Godin 1988). For example, swimming and pursuit speeds and prey pursuit (or reaction) distances increased with fish size in the three species of cyprinids observed by Wanzenböck and Schiemer (1989), as is the case for other species and for golden shiner larvae. Although such size-related changes in foraging behaviour have been reported for many fish species, these parameters have only recently been interpreted with the goal of determining the fish's search strategy (Browman and O'Brien 1992). In the sections that follow, we examine the behavioural characteristics of each component of the golden shiner larvae predation cycle and compare them with those that would be exhibited by a saltatory searcher.

Do Shiner Larvae Search Only While Stationary?

Saltatory searchers scan for prey only during the frequent stationary pauses between repositioning movements (O'Brien et al. 1986, 1989). Several lines of evidence support this interpretation (see O'Brien et al. 1990).

A characteristic distinguishing saltatory from cruise or ambush foragers is the distribution of pursuits within the search space. Assuming that they simultaneously scan the entire search space, both cruise and ambush searchers would be expected to pursue prey at the boundary of the search space. Saltatory-searching white crappie larvae and juveniles pursue prey throughout the search space (O'Brien et al. 1986, 1989; Browman and O'Brien 1992). So do golden shiner larvae (Fig. 3).

Pauses between swimming movements may have functions other than search, e.g. time to orient toward a prey located while swimming or to initiate the attack phase of the predation cycle. If either of these were the case, all or most pauses would be followed by attacks on prey. This is never the case. Only 10–25% of all pauses by white crappie juveniles are followed by attacks on prey (O'Brien et al. 1986). For white crappie larvae, this figure is 1–10% (Browman and O'Brien 1992), as is the case for golden shiner larvae. Further, in every case in which prey were located, orientation toward it did not occur during the stationary pause, but immediately after it, while the fish was engaged in its pursuit of the prey item. Although it is impossible to exclude other possibilities, these observations are consistent with the saltatory-search hypothesis and we conclude that golden shiner larvae search for prey only while stationary.

Drost and van den Boogaart (1986) reported that carp (*Cyprinus carpio*) larvae punctuate their swimming movements with periods of rest that average 0.3 s. The duration of these "rest" periods is similar to the stationary search times reported here for shiner larvae and elsewhere for white crappie larvae (Browman and O'Brien 1992). We suggest that such commonly observed stationary periods, which have been interpreted as times of rest, may in some cases be associated with prey search.

Relationship between Movement Pattern and Search Space Geometry

Animals project what can be termed a scan space while foraging. The geometry of this space is species specific and plastic, varying with environmental conditions and prey type (O'Brien 1979; Dunbrack and Dill 1984; Evans and O'Brien 1988; O'Brien et al. 1989; Bell 1990). For saltatory-searching fish, which are not searching while moving, swimming movements between stationary pauses serve only to bring the animal into a volume of water that has, for the most part, not previously been scanned. If this is indeed the case, there should be a relationship between the distance and angle traveled in repositioning moves and the geometry of the space previously scanned. For example, if after an unsuccessful search an animal moves only a small distance relative to the maximum distance to which it can locate prey, much of its search volume will be scanned again (see O'Brien et al. 1989). Similarly, at least when prey are abundant, it would be energetically wasteful for an animal to move a distance that would take it far beyond the search space it had just scanned.

Turning after a stationary pause is another way saltatory searchers can quickly move into unsearched areas. To benefit from turning (in terms of yield of unsearched space), a saltatory-searching fish must make wider turns in response to larger pursuit angles (O'Brien et al. 1990).

For golden shiner larvae, maximum pursuit distance increased with fish size and, on average, move distances were slightly shorter (Table 1; Fig. 2 and 3). Further, most pursuits occurred at angles of 20–60° from forward-directed (Fig. 3); there is a pronounced lack of pursuits in the first 0–20° of the search space. The distribution of move angles is similar (Fig. 2), indicating that golden shiner larvae modulate their swimming movements in the same manner as do other saltatory searchers, i.e. to minimize redundant search efforts (O'Brien et al. 1989).

Conclusions and General Considerations

Our results indicate that at least some fish larvae are not cruise searchers. Rather, the search pattern employed by golden shiner larvae, and that of white crappie larvae (Browman and O'Brien 1992), is much like the saltatory-search strategy that we have described for juvenile zooplanktivores. Further, in recent experiments, one of us (Browman) has observed similar search patterns in Atlantic cod (*Gadus morhua*) larvae.

Accurate characterization of a fish's search pattern is important for measuring and interpreting, among other things, predatory-prey encounter frequencies, prey choice and dietary preferences, and the efficiency of movement patterns associated with foraging. For example, virtually all characterizations of search strategies in fish larvae imply that they are cruise searchers (e.g. Rosenthal and Hempel 1970; Hunter 1972; Wanzenböck and Schiemer 1989; Arnold and Holford 1990). Specific examples include the description of herring larvae as "tube searchers" that swim through the water projecting a tube-

shaped search space (Rosenthal 1969; Rosenthal and Hempel 1970). This characterization implies cruise search. Similarly, plaice (*Pseudopleuronectes platessa*) larvae have been described as projecting an elliptical search space as they swim through the water column (Arnold and Holford 1990). Further, in simulation models of foraging in fish larvae, it is common practice to calculate search rate (or the volume of water scanned) as the product of mean (continuous) swimming speed and the cross-sectional area of the perceptual field (e.g. Wanzenböck and Schiemer 1989; Arnold and Holford 1990; also see review in Blaxter 1986). These search rates, along with estimates of prey abundance, are used to establish prey encounter rates for the larvae. Such calculations assume that the fish is searching continuously as it swims: a cruise search strategy is implied.

For a saltatory-searching fish, which searches only while stationary, the calculation of prey encounter rate described above will not accurately reflect the true rate. Rather, it is the mean duration of the stationary periods that punctuate swimming movements and their frequency that should be used to estimate search time. Using a simulation model currently under development, we are quantitatively evaluating differences between pause-travel and cruise search interpretations of search patterns in fish larvae.

Acknowledgments

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Commercial Troll Fishing Vessel Distribution off Vancouver Island during July 1988: Relation to Observed Physical Oceanography

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We compare distributions of salmon troll fishing vessels obtained using navigational radar observations off southwestern Vancouver Island in mid-July 1988 with the circulation and water property structure derived from concurrent oceanic measurements. Results support the hypothesis that vessels aggregate in clusters within the transition zone formed between the upwelling-induced shelf-break current and buoyancy-driven Vancouver Island coastal current. Dense clusters of vessels within this zone appear to be coincident with mesoscale (10 km) circulation features embedded in the large-scale (100 km) coastal upwelling regime. In all instances, the association of vessel aggregations and oceanic circulation illustrates a response of the salmon to local oceanographic conditions. The circulation features presumably correspond to upwelling-enriched regions with high food organism densities that attract aggregations of salmon.

Nous avons comparé les distributions des bateaux de pêche au saumon à la ligne traînante à l'aide d'observations radar de navigation au large du sud-ouest de l'île de Vancouver, vers la mi-juillet de 1988, avec des caractéristiques de circulation et de composition de l'eau provenant de mesures océaniques simultanées. Les résultats supportent l'hypothèse selon laquelle les vaisseaux se regroupent en flottilles dans une zone de transition formée entre le courant de la zone de rupture induit par la remontée d'eau et le courant côtier de l'île de Vancouver créé par la flottation. Les regroupements les plus denses de vaisseaux dans cette zone semblent coïncider avec les caractéristiques de circulation à l'échelle méso (10 km) faisant partie du régime de remontée d'eau côtier à grande échelle (100 km). Dans tous les cas, l'association des vaisseaux de pêche et de la circulation océanique illustre une réponse du saumon aux conditions océanographiques locales. Les caractéristiques de circulation correspondent probablement aux régions enrichies par des remontées d'eau contenant de fortes densités d'organismes nutritifs, qui attirent les bancs de saumons.

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Healey et al. (1990) found that the aggregation of salmon troll fishing vessels off the southwest coast of Vancouver Island was tied closely to the prevailing circulation pattern. Concentrations of vessels in the summers of 1982 and 1983 were coincident with (1) the transition zone over the mid-continental shelf that separates the buoyancy-driven Vancouver Island coastal current from the upwelling-driven shelf-break current, (2) the margins of the upwelling-induced cyclonic (anticlockwise) eddy situated over Juan de Fuca Canyon off the mouth of Juan de Fuca Strait, and (3) nearshore regions of enhanced tidal mixing and rectified tidal flow. A principal shortcoming of Healey et al. (1990) was the lack of physical oceanographic data for the time of the fishboat aggregation measurements. Findings were based primarily on a knowledge of the typical circulation patterns established in the region in summer, supplemented by thermal imagery for 1982 and 1983 from the Advanced Very High Resolution Radiometer

(AVHRR) on the National Oceanic and Atmospheric Administration (NOAA) series of satellites. As a consequence, details of vessel distributions could not be unequivocally related to the regional oceanography. A further aspect of the fishboat distributions was the occurrence of dense, highly localized aggregations of fishing vessels within the regions of high vessel concentration. In the absence of coincident vessel distribution and oceanographic data, Healey et al. (1990) could only speculate on the reasons for the dense vessel clusters.

A survey program was conducted in 1988 to confirm the link between troll fishboat distribution and prevailing circulation over the southwest British Columbia shelf during the main fishing period in July. According to Healey et al. (1990), the band of vessels that parallels the coast north of La Perouse Bank in summer is located within an upwelling-induced "oceanic ridge" (transition zone) which separates the Vancouver Island coastal current from the shelf-break current. A further objective



FIG. 1. Map of the survey region. Broken line is the international Canada-USA fishing boundary. Depth contours are in metres. Triangles give the locations of moored current meters during July 1988.

of the 1988 study was to test the hypothesis that dense, localized concentrations of vessels found over midshelf are linked to pockets of intensified upwelling embedded in the prevailing upwelling regime. Presumably formed through meandering and eddy formation in the southeastward flowing shelf-break current, these mesoscale features appear to be regions of high productivity which attract large aggregations of fish.

Methods

Troll Fleet Distributions

As in Healey et al. (1990), trolling vessel distributions were derived from photographs of the radar screen at the Canadian Coast Guard navigational radar station at Ucluelet (Fig. 1). The radar sweeps an arc of about 90 km radius covering the area from the entrance to Juan de Fuca Strait to Estevan Point. For this study, radar images of small vessel targets were collected twice daily (morning and afternoon) over the period July 17–21 coincident with a coastal oceanographic survey from the CSS *Parizeau*. Because the radar does not “see” all of the boats all of the time, our sampling provided a conservative estimate of the actual boats in the fishing areas on a given day.

Further details on the method can be found in Healey et al. (1990).

Information on the composition of the catch by individual vessels fishing in specific locations was available from the troll logbook program administered by the Department of Fisheries and Oceans, Biological Sciences Branch, Nanaimo, B.C. (Jordan and Carter 1987). Catch records for the July 17–20 period provided information on the rate of capture of different salmon species by vessels associated with particular vessel aggregations.

Water Property Observations

The regional distribution of water properties within the study area was derived from vertical profiles of temperature, salinity, and dissolved oxygen taken along six cross-shelf survey lines (Fig. 2). Detailed maps of water properties in the vicinity of two major fishboat aggregations were obtained by altering the daytime schedule of the ship survey according to updated knowledge of the fishing fleet distribution. Each morning, a coded message giving the locations and relative intensities of the vessel aggregations was sent to the survey ship from the radar station. The ship then proceeded to a given site for closely spaced sampling. Sites were chosen on the basis of vessel den-

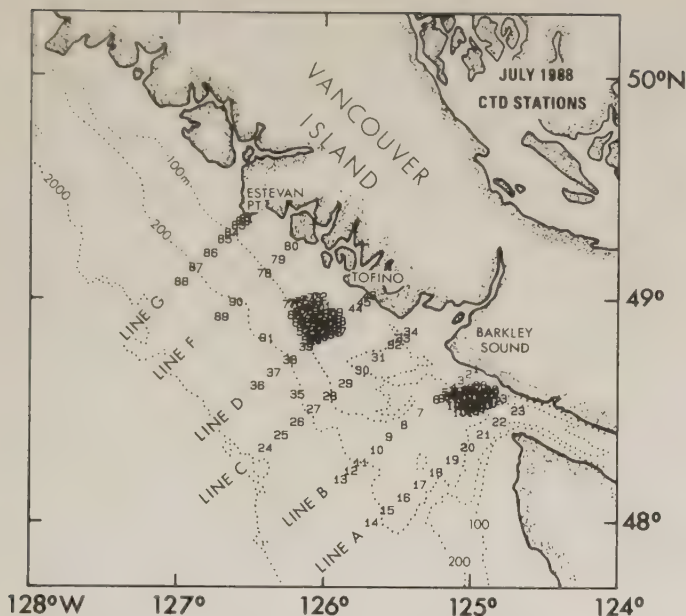


FIG. 2. Location and cast numbers of CTD/hydro stations occupied from July 15 to 20, 1988. Dense clusters of stations correspond to regions that had major fishboat concentrations.

sity and proximity to the ship. Because of time constraints, we were able to sample only two aggregation sites during the 4-d cruise period (Fig. 2).

Water property profiles were obtained using a Guildline digital conductivity-temperature-depth (CTD) sensor and 1.5-L Niskin bottles. The oceanographic stations for the regional survey along lines A-G were spaced at roughly 25- to 35-km intervals in the longshore direction and 5- to 10-km intervals in the cross-shelf direction (Fig. 2). Oceanographic stations for the two fine-scale surveys in the vicinity of fishboat concentrations were spaced at 5-km intervals in the longshore direction and 3-km intervals in the cross-shore direction. The northern small-scale survey consisted of 31 stations centered near midshelf in about 110 m of water approximately 30 km southwest of Tofino (fishboat cluster 2, see Fig. 3); the southern survey consisted of 22 stations centered over Swiftsure Bank in about 100 m of water near the entrance to Juan de Fuca Strait (cluster 5, Fig. 3). Dissolved oxygen measurements were taken at most of the regional grid stations but at only five of the fine-scale stations.

Clear skies and negligible coastal fog enabled us to obtain usable AVHRR imagery for much of the region throughout the field program. When corrected for geometric distortion and empirically calibrated using in situ temperature measurements, these data provide a measure of the sea surface temperature at spatial pixel resolutions of 1.1 km. We also created quantitative maps showing the location and intensity of horizontal temperature gradients in the study region. In particular, each pixel of each of the original AVHRR images was remapped as the magnitude of the temperature variance in a 5×5 pixel² (5.5×5.5 km²) window centered on that pixel. Fronts and regions of nonuniform temperature distribution are particularly well defined by this procedure. Hourly records of current velocity at depths of 35 and 100 m were obtained from current meter moorings A1, B1, AB1, and J1 (Fig. 1) located over the southern shelf as part of the "La Perouse Bank Project" (cf. Ware and Thomson 1990). Surface current data also were obtained from mooring E1 (Fig. 1) deployed to the southwest of Estevan Point to monitor the presence of the Vancouver Island coastal current. Maps of geostrophic currents were obtained using the

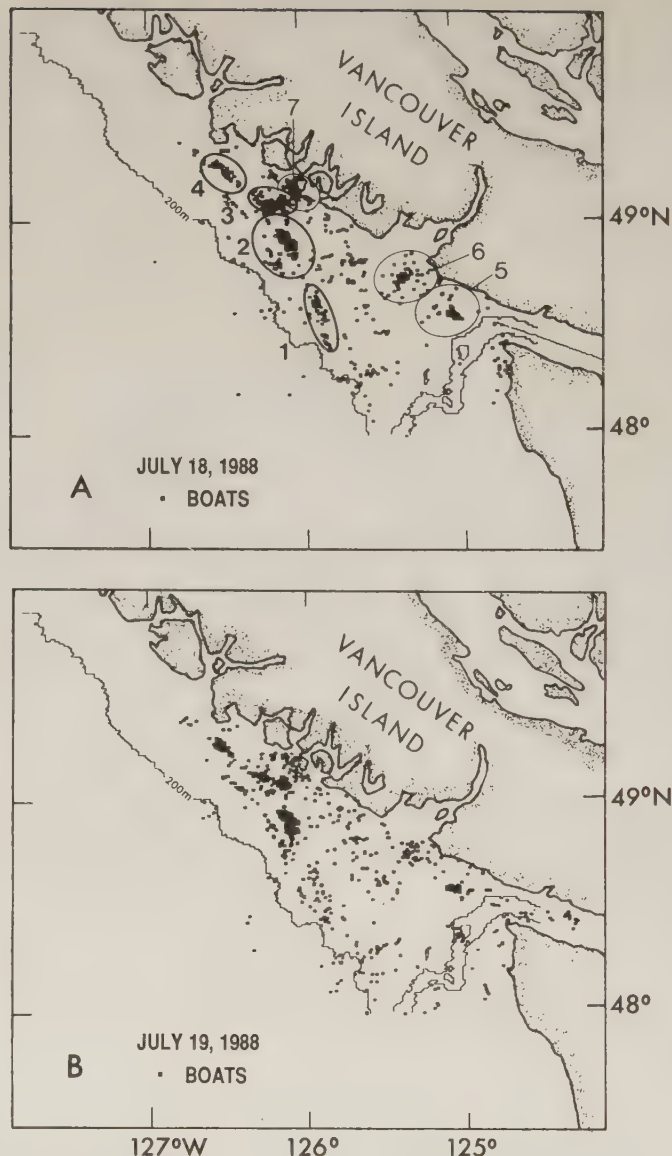


FIG. 3. Distribution of troll fishing vessels (squares) during July 1988. (A) July 18, a.m. and p.m. combined; (B) July 19, a.m. and p.m. combined. Solid line is the 200-m depth contour.

geopotential surfaces (dynamic heights) derived from the integrated density distributions.

Acoustic Targets

We used the ship's 12-kHz echo sounder to gather information on the occurrence of fish-like targets along sections of the survey tracks in Fig. 2. Acoustic records were available for most of the survey lines north of Barkley Sound. Transects not included were lines A and B, stations 24-30 on transect C, the track between stations 34 and 36 (inshore end of line C to the seaward end of line D), and the track between stations 71 and 74 along the northernmost boundary in the northern fine-scale survey. Two types of targets were identified on the acoustic records: dense, arrow-shaped targets that could be counted individually and an acoustic scattering layer that could only be assessed qualitatively. Using a standard "rule of thumb" that a sounder can distinguish individual targets larger than about one quarter of a wavelength (D. Mackas, DFO, Institute of Ocean Sciences, Sidney, B.C., pers. comm.), the arrow-shaped

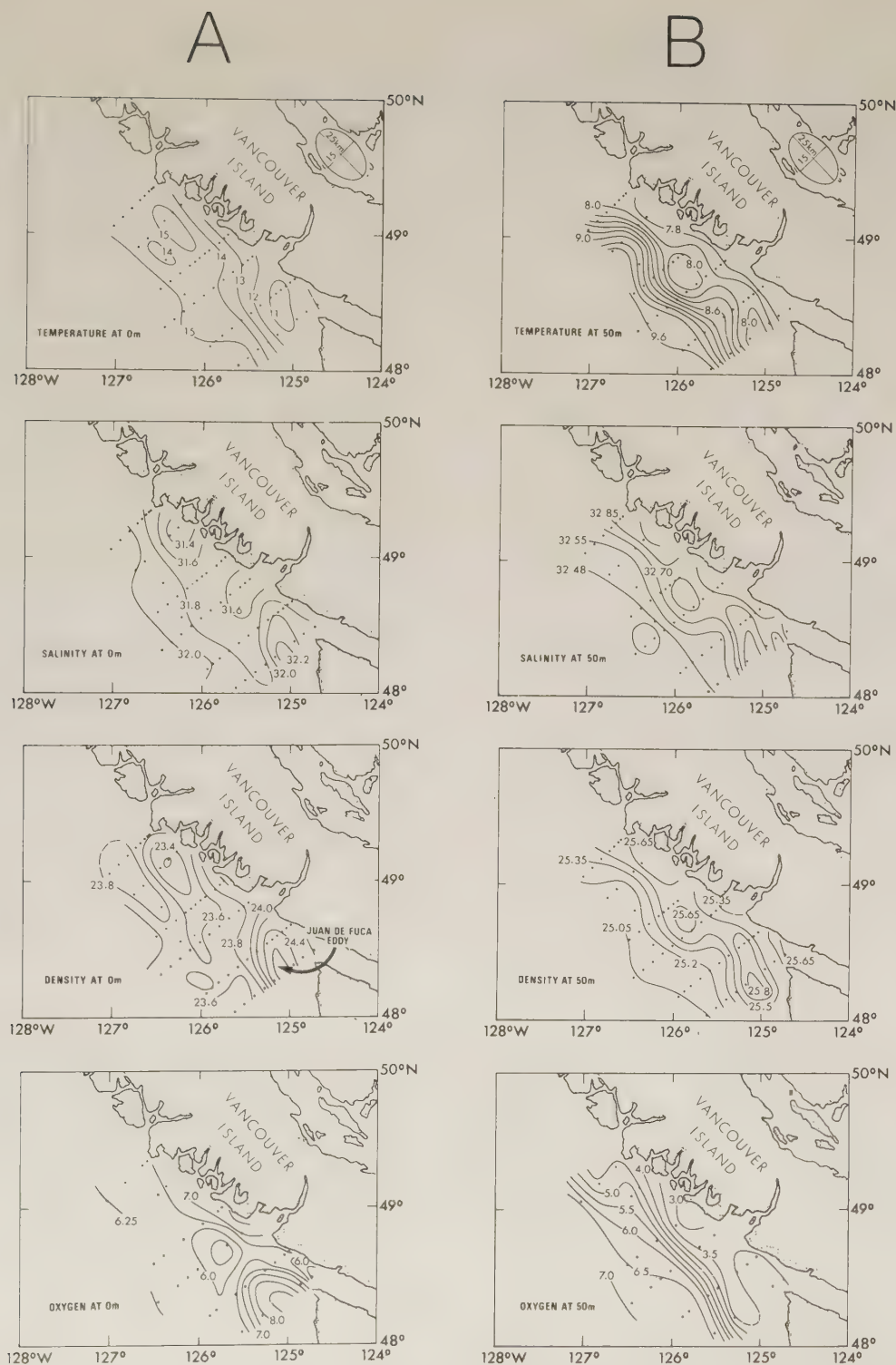


FIG. 5. Distributions of temperature ($^{\circ}\text{C}$), salinity (ppt), density (σ_t), and dissolved oxygen (mL/L) at selected depths. (A) Surface (0 m); (B) 50 m. Cross-shore and alongshore correlation scales of 15 and 25 km have been assumed for the contour mapping (except for the sparsely sampled oxygen data for which the scales are 25 and 50 km).

July 16 along line A and ending July 20 along line G (Fig. 2). For the 15- and 25-km correlation scales used for the regional objective mapping, inclusion of the high-density CTD grids did not significantly modify the distributions. Except for the region occupied by the Juan de Fuca Eddy to the west of Juan de Fuca Strait, the upper layer (Fig. 5A) was characterized by warm, high-salinity water in the off-

shore region and cool, low-salinity water in the nearshore region. The subsurface waters (Fig. 5B) consisted of warm, low-salinity, high-oxygen water in the offshore region and cool, high-salinity, low-oxygen water in the nearshore region. Relatively cold, salty, high-density water was found in a midshelf ridge extending from roughly 48.2°N , 125°W to 49°N , 126°W .

The observed structure in the subsurface water properties over the slope and outer shelf in July 1988 was the result of wind-induced upwelling of cold, high-salinity, low-oxygen slope water across the shelf-break (200-m depth contour); the structure of the inner shelf water arose from the nearshore northwestward flux of cold brackish surface water exiting Juan de Fuca Strait (Thomson et al. 1989; Griffin and LeBlond 1990; Hickey et al. 1991). Upwelled water found within the core of the quasi-permanent Juan de Fuca Eddy near 48.3°N, 125°W (Fig. 5B) originated with pressure-induced subsurface transport through Juan de Fuca Canyon onto the southern shelf via a small spur canyon (Freeland and Denman 1982). The meso-scale cyclonic eddy centered near 48.7°N, 126°W in July 1988 (Fig. 5B) was presumably a transient feature arising through meandering or instability of the longshore flow (cf. Thomson 1984; Thomson and Gower 1985). Such partially isolated regions of low-temperature, high-salinity (high density) water over the outer shelf represent zones of comparatively intense cyclonic flow and intensified upwelling over the continental margin.

For the region as a whole, the combined effect of wind-induced upwelling and northwestward flux of low-density water is to produce a "doming" of the subsurface isopycnals over the central portion of the shelf (Thomson et al. 1989; Healey et al. 1990). This ridge-like domain, with minimum isopycnal depth over midshelf, is clearly defined in the depths of the 25.0- and 25.6- σ_t surfaces for July 1988 (Fig. 6). The degree of doming, and hence large-scale upwelling, was not uniform along the coast but varied over alongshore scales of order 100 km. There was also a low-frequency component to the oceanic variability which we are not able to resolve in our 5-d data set but which was evident in the current meter records. In the present case, upwelling was most intense in the cores of the Juan de Fuca Eddy and the transient eddy centered near 48.7°N, 126.0°W. Regions of weak upwelling were found between these

two features. Comparison of the fishboat distributions in Fig. 3 with the water property structure in Fig. 5 and 6 reveals that the four midshelf vessel clusters (clusters 1–4) were located within the core of the ridge domain. Nearshore clusters 5–7 were located landward of the ridge domain.

Based on the moored current meter records and the geostrophic current pattern derived from the observed density field (not shown), the prevailing flow pattern at the time of the July 1988 survey was similar to that discussed in Healey et al. (1990). In particular, prevailing currents over the slope and outer shelf were dominated by the southeastward flowing shelf-break current whereas prevailing currents over the inner shelf were dominated by the northwestward flowing buoyancy-driven Vancouver Island coastal current (cf. Freeland et al. 1984; Thomson et al. 1989; Hickey et al. 1991). The two current regimes were separated by a transition zone (the ridge domain) of weak circulation. Although the half-hourly current meter time series off Estevan Point indicated a temporary (wind-induced) reversal in the coastal current during July 18–20, the flow prior to, and after, this period was to the northwest at speeds of 10–20 cm/s, consistent with the typical summertime flow pattern (M. Woodward, DFO, Institute of Ocean Sciences, Sidney, B.C., pers. comm.).

Local Water Property Structure and Circulation

The detailed water property survey conducted in the immediate vicinity of the two fishing vessel aggregations (Fig. 7 and 8) revealed an underlying spatial structure not resolved by the regional distribution maps. The northern grid (Fig. 7) was dominated by a localized zone of upwelled water approximately 10 km wide and over 10 km long situated near the northern boundary of the cyclonic eddy centered at 48.7°N, 126°W (Fig. 5B). Isopycnals in the core of this feature were 10 m shallower than those of the surrounding region and penetrated from near-bottom (100 m depth) to about the 10-m depth level in the

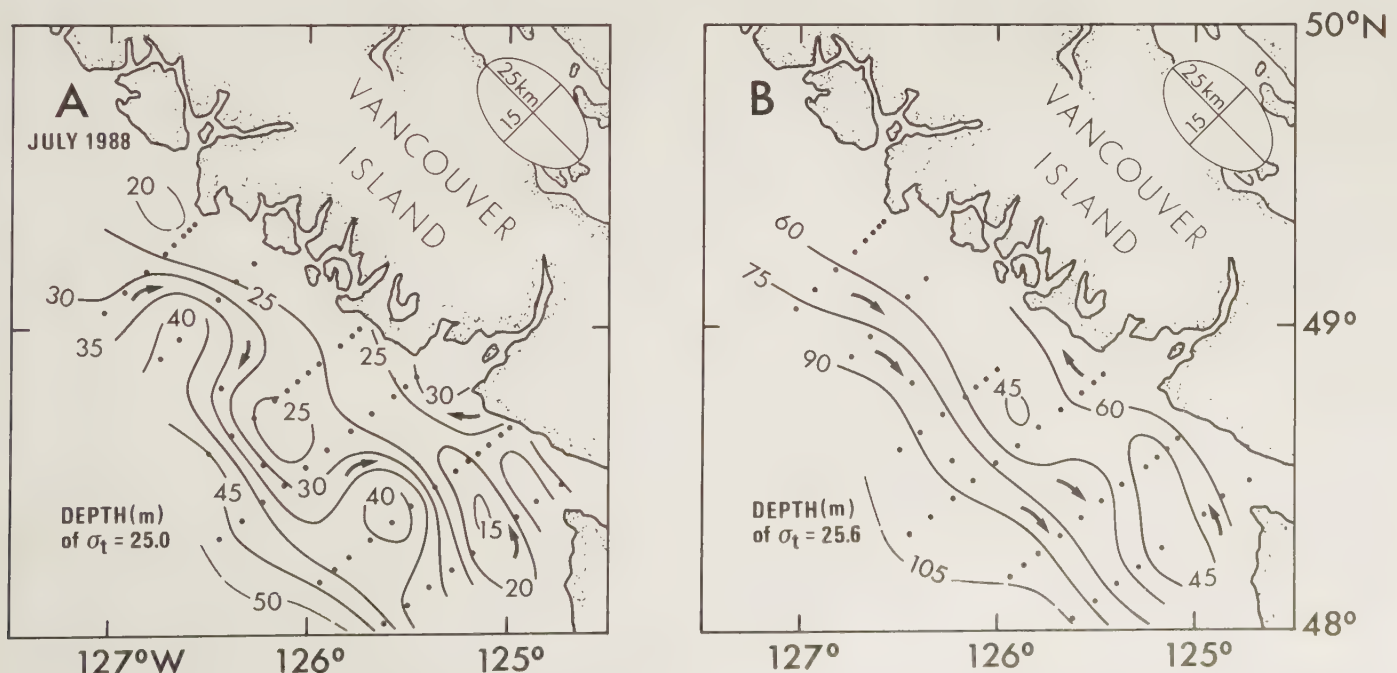


FIG. 6. Depths of isopycnal surfaces. (A) 25.0 σ_t surface; (B) 25.6 σ_t surface. Ellipses give mapping correlation scales (see Fig. 5). The "ridge domain" occupies the longshore region of relatively shallow isopycnal depths. Arrows denote the flow direction for the prevailing surface current.

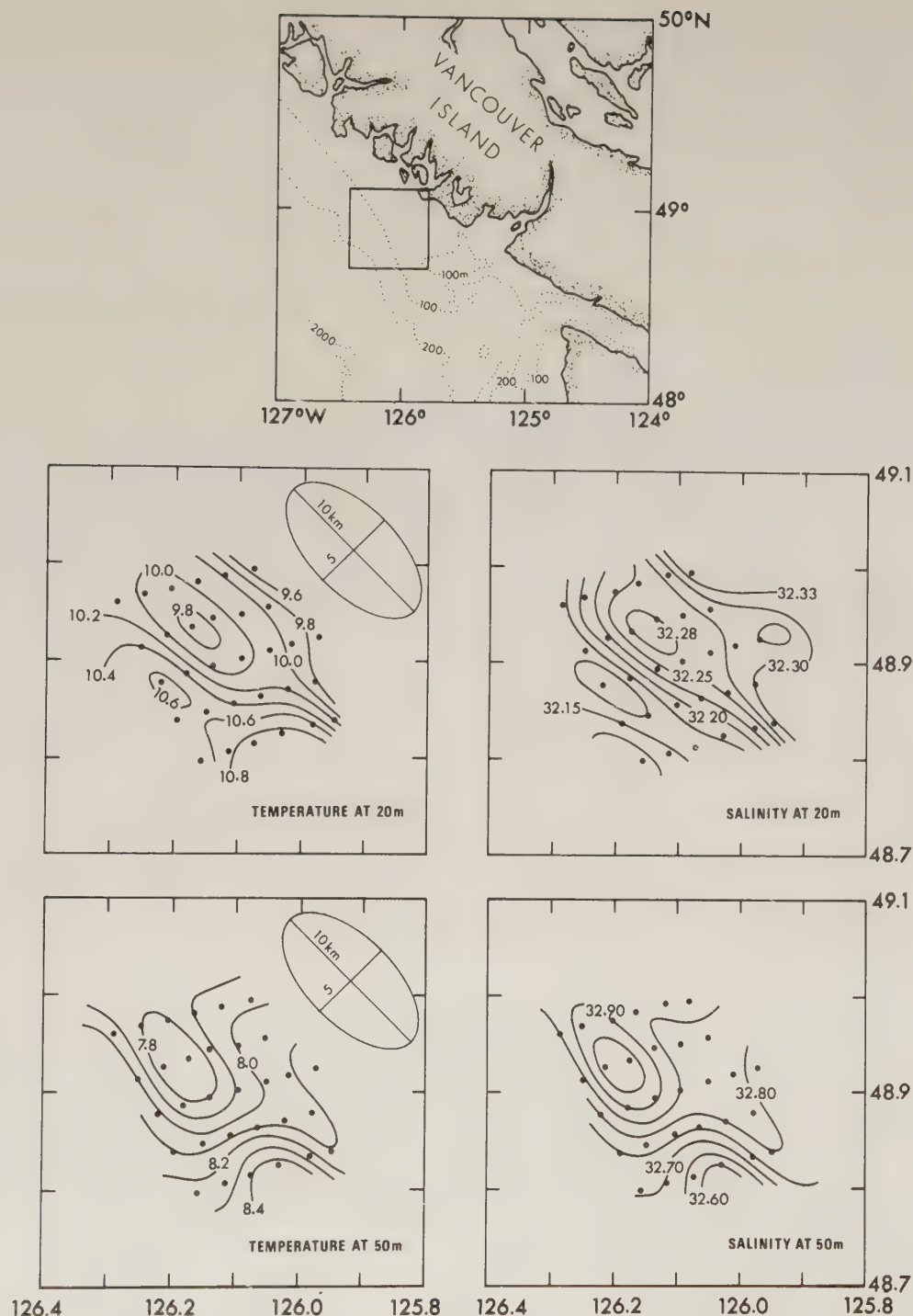


FIG. 7. Temperature and salinity contours at depths of 20 and 50 m for the northern fine-grid CTD survey. Ellipses denote correlation scales used in the mapping.

water column. Dissolved oxygen data (not shown) indicate the presence of upwelled water over the northern sector of the grid, but the sampling was not sufficiently dense to define the central portion of the upwelling region. Most of the fishboats in the survey area were located along the eastern side of the upwelling core; a small string of boats was located along the western side of the upwelling core (Fig. 3 and 7).

The area covered by the three CTD lines in Fig. 8 was limited but sufficient to reveal a region of warm, low-salinity (low-density) water over the southwestern tip of Swiftsure Bank. Isopycnals in this localized downwelling region were depressed over 10 m relative to isopycnals over the southeastern edge of

the bank. This feature appears to be associated with strong topographic "steering" (deflection) of the subsurface flow exiting Juan de Fuca Strait. In this case, the fishboats were congregated mainly to the north of the eddy core (compare Fig. 3 and 8).

Geostrophic currents (Fig. 9) for the two small-scale CTD grids were derived from the observed dynamic height (integrated density) fields. These flows represent time-averaged, quasi-synoptic estimates of the nontidal component of the surface currents relative to the assumed depth-of-no-motion of 50 m. The approximately 0.10 m/s southeasterly flow over the northern grid (Fig. 9A) indicates that the region was along the shoreward boundary of the shelf-break current. However, the

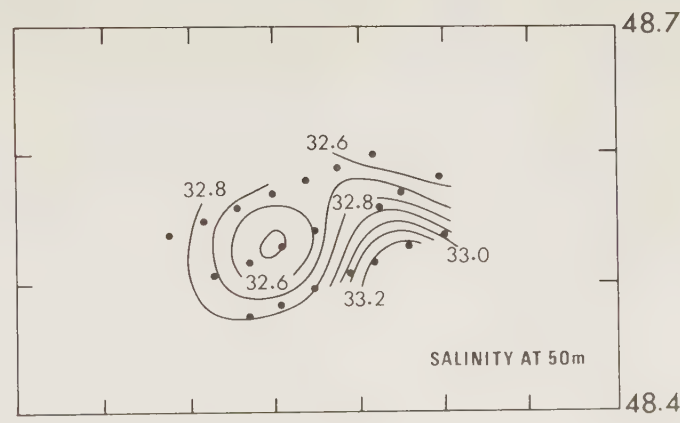
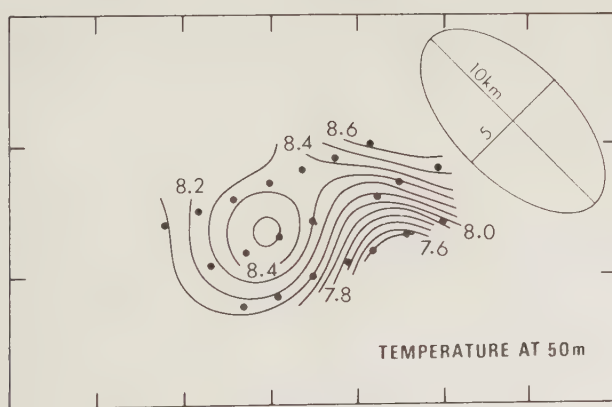
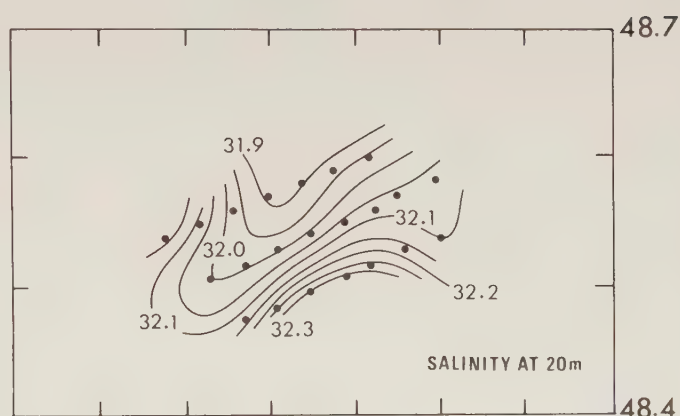
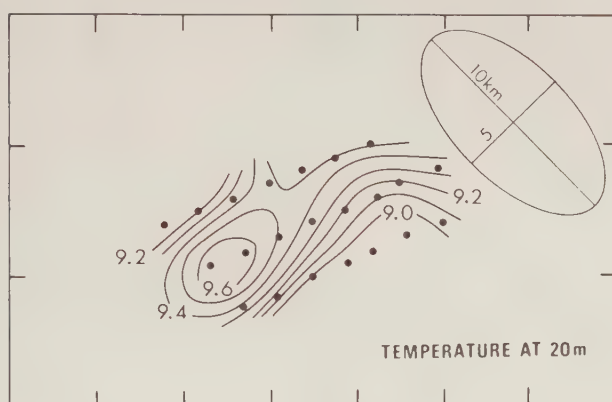
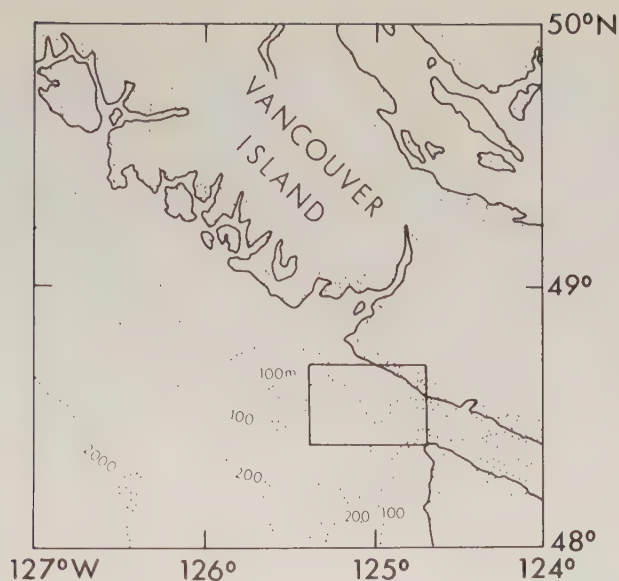


FIG. 8. Same as Fig. 7 but for Swiftsure Bank.

shelf-break flow was not as uniform as regional geostrophic current maps such as those in Healey et al. (1990) would suggest but was split into distinct branches in the vicinity of the upwelling center. Circulation near the upwelling center was counterclockwise with a region of near-zero horizontal velocity near its core. Over Swiftsure Bank (Fig. 9B), the situation was considerably different. Here, the approximately 0.10 m/s flow was steered around the boundary of the bank by the bottom topography to form a strong westward flow along the southern flank of the bank and a downwelling regime over the central portion of the bank.

Relationship of Fishboat Distribution to Thermal Imagery

The correspondence between vessel congregations and the regional physical oceanography is further illustrated by comparison of the vessel distributions with AVHRR thermal imagery (Fig. 10). Fishboat clusters along midshelf occupied a corridor of warm surface water bordered on either side by regions of colder water. This cross-shelf transition from cool outer-shelf surface water, through warm midshelf water, to cool inner-shelf surface water coincided with the transition from the

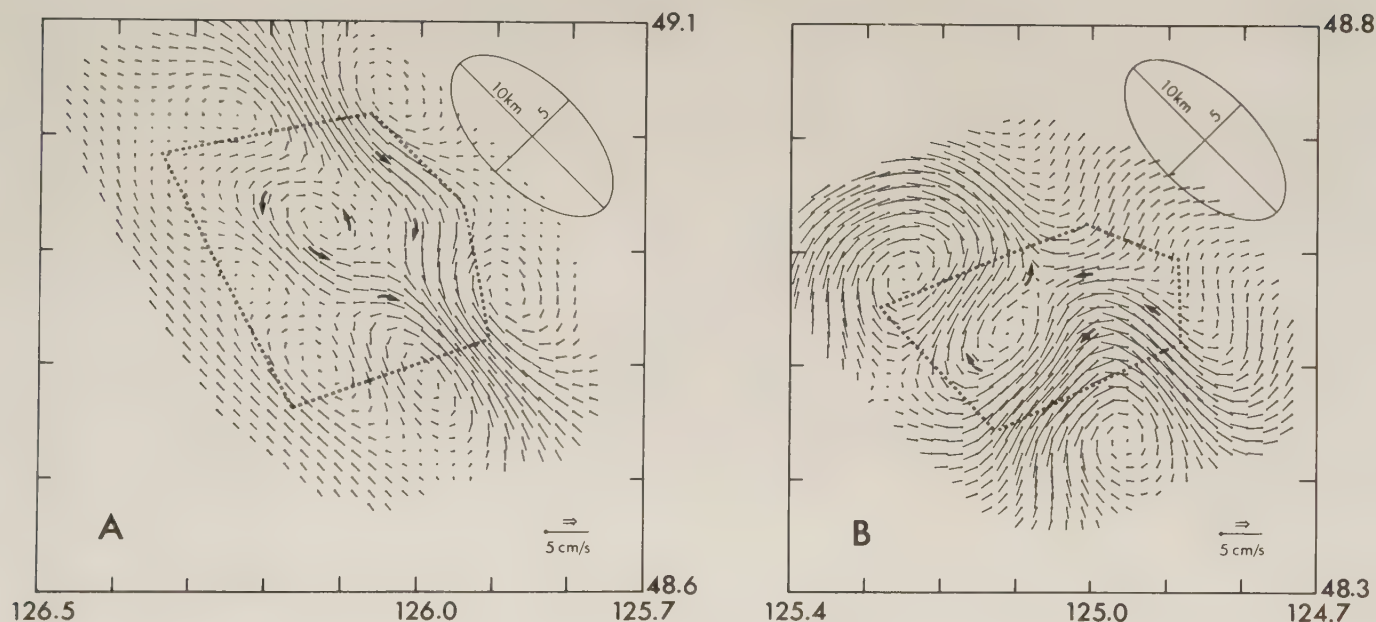
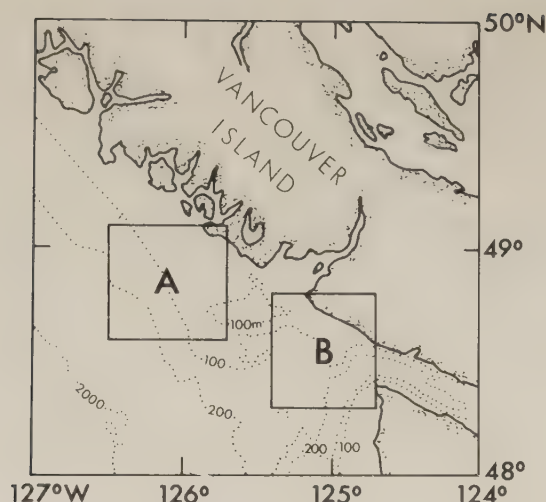


FIG. 9. Surface geostrophic currents relative to 50 m depth for the fine-grid surveys. Ellipses denote mapping correlation scales. (A) Northern grid region; (B) Swiftsure Bank region. Only contours within the broken box are considered reliable.

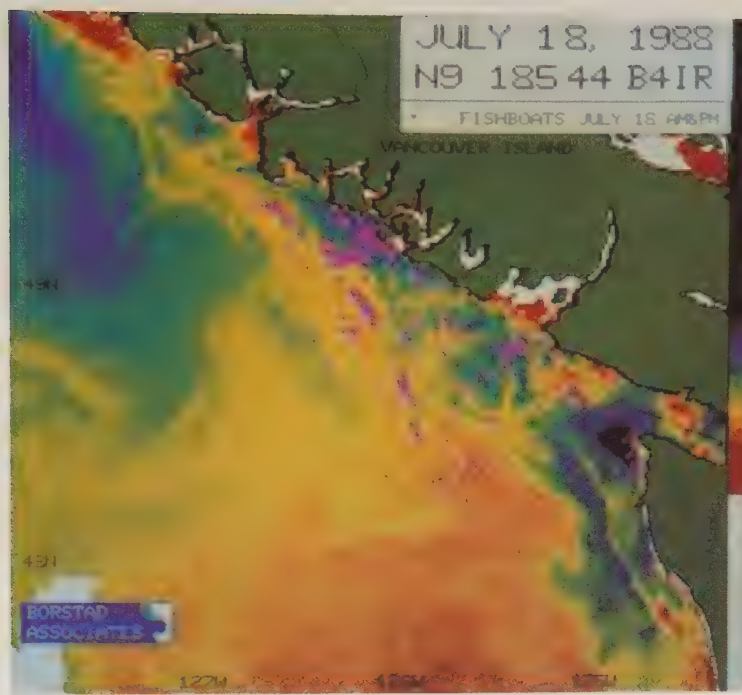
southeastward flowing shelf-break current to the northwestward flowing coastal current. A majority of vessels were found in regions with comparatively weak thermal variance (Fig. 11), similar to what was observed in 1983 (Healey et al. 1990). However, if we ignore variance regions which had only one vessel (to avoid contributions from outliers and vessels in transit), the regional concentration of vessels in 1988 was not inversely related to thermal variance as it was in 1983 but reached near-uniform values of $0.02\text{--}0.03\text{ boats/km}^2$ in the variance range $5\text{--}20(^{\circ}\text{C})^2$ (Fig. 11). Low vessel concentrations were observed at the lowest variance levels ($0\text{--}1(^{\circ}\text{C})^2$). At variances greater than $20(^{\circ}\text{C})^2$, vessel concentrations (based on very few vessels) tended to increase with increased variance.

The majority of fishboats over the inner shelf (water depths $1\text{--}60\text{ m}$) had peak concentrations of $0.03\text{--}0.04\text{ boats/km}^2$ in the $4\text{--}7(^{\circ}\text{C})^2$ variance range but, for the most part, were distributed randomly throughout the variance range (Fig. 12A). This presumably represents boats fishing in active tidal mixing zones near coastal promontories and over shallow banks in close proximity to the coast (M. Foreman, DFO, Institute of Ocean Sciences, Sidney, B.C., pers. comm.). The vessels in the mid-

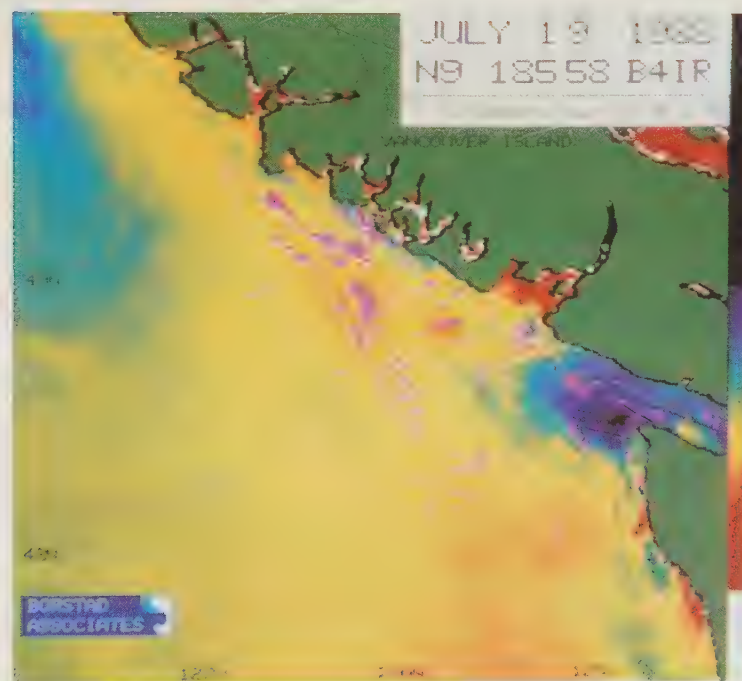
shelf depth range of $61\text{--}100\text{ m}$, where most vessels congregated, attained peak concentrations of $0.04\text{--}0.06\text{ boats/km}^2$ in the thermal variance range $1\text{--}6(^{\circ}\text{C})^2$ (Fig. 12B) and accounted for the peak concentrations in the overall distribution for July 19 (Fig. 11B). The concentration of 0.07 boats/km^2 in this depth range was the highest value observed in the region. Low fishboat concentrations were observed at zero variance. Over the remaining portion of the continental margin (depth ranges $101\text{--}200$ and $201\text{--}1000\text{ m}$), most boats were again located in regions of low thermal variance, with almost all the fishboats seaward of the shelf break, preferring regions of low thermal structure (Fig. 12C and 12D).

Discussion

The survey results for July 1988 support the hypothesis (Healey et al. 1990) that troll fishing vessels cluster within the midshelf transition zone that separates the buoyancy-driven Vancouver Island coastal current (inner shelf) from the upwelling-induced shelf-break current (outer shelf). The prevailing flow was strongly directed by the bathymetry, and the overall



A



B

FIG. 10. Radar-derived fishboat positions (squares) superimposed on the coincident NOAA AVHRR satellite imagery. (A) July 18, a.m. and p.m.; (B) July 19, a.m. and p.m. Temperatures in Fig. 10A range from 11.3 (dark blue) to 17.8°C (dark red) in increments of 0.5°C and in Fig. 10B range from 10.2 to 21.7°C.

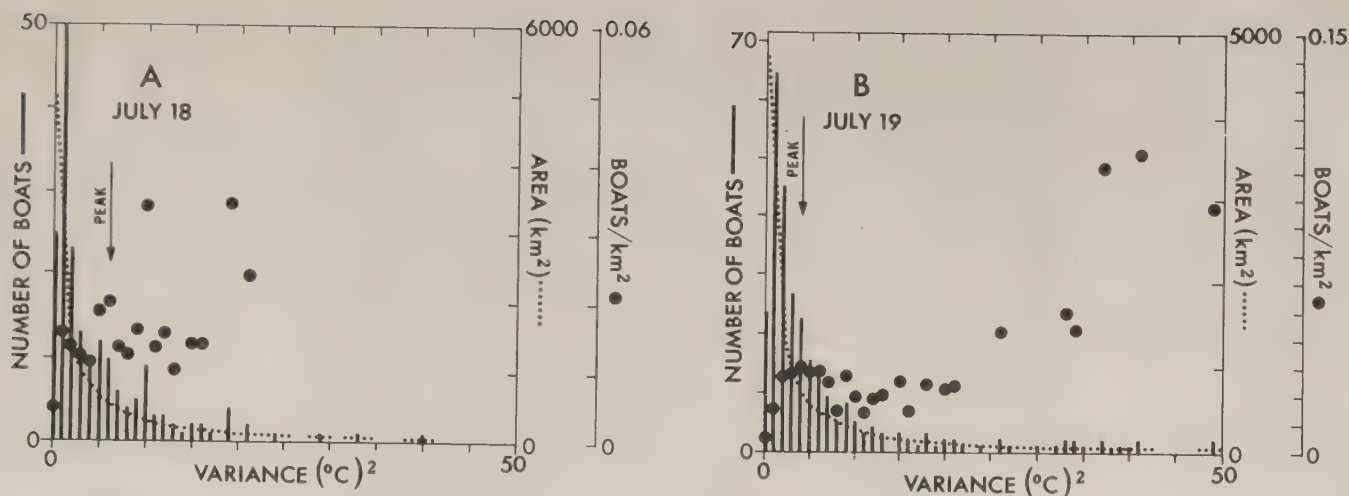


FIG. 11. Fishboat distribution versus temperature variance for all vessels. (A) July 18, p.m.; (B) July 19, p.m. Plots give the number of vessels in each variance range (vertical bars), the area of ocean covered by each variance range (dotted line), and the density of vessels in each variance range (large dots). Density values for single vessels are not plotted.

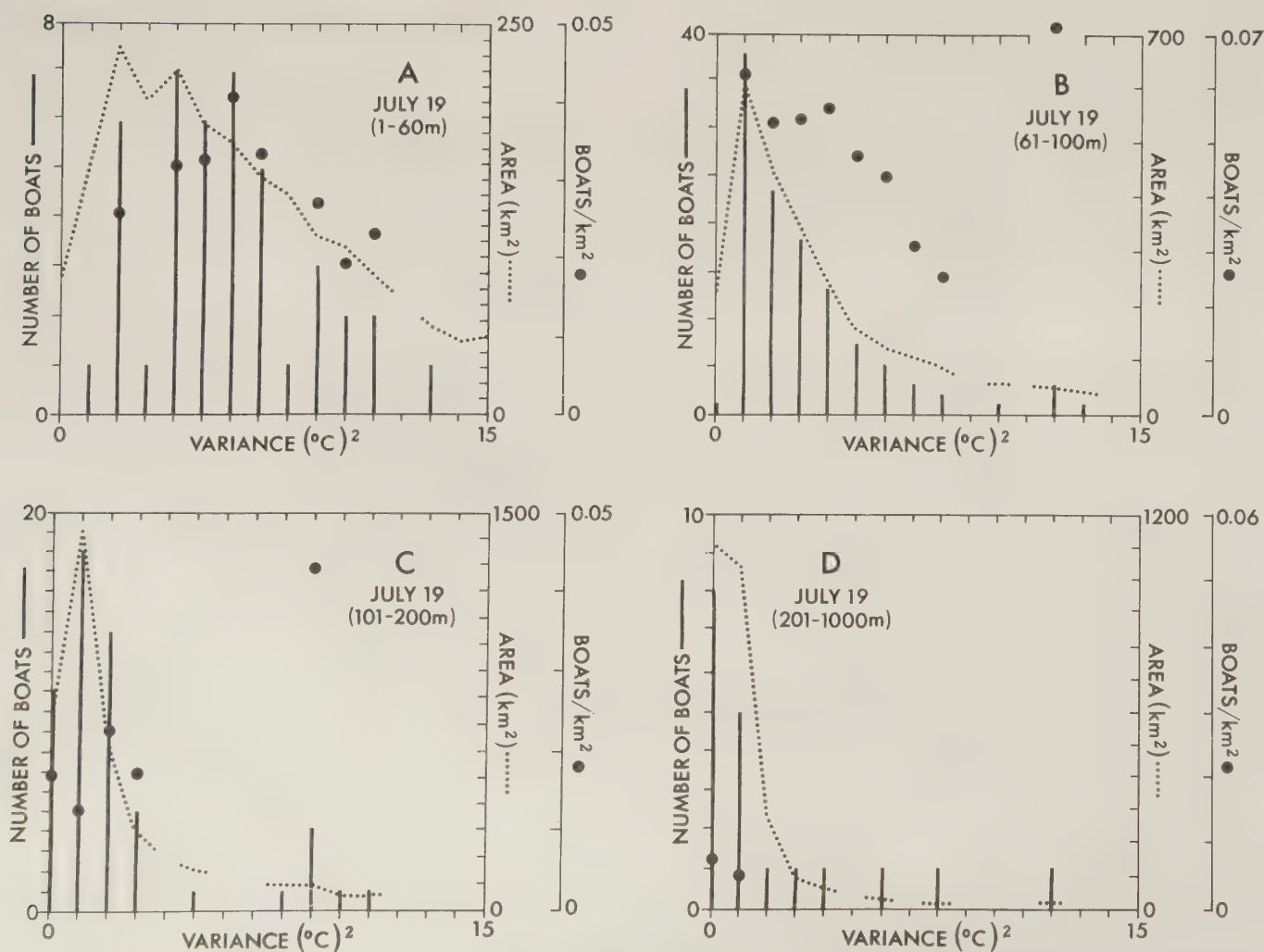


FIG. 12. Fishboat distributions for July 19 (p.m.) partitioned according to water depth. (A) 0-60 m; (B) 61-100 m; (C) 101-200 m; (D) 201-1000 m. Symbols are the same as in Fig. 11.

distribution of vessels was banded parallel to the 100-m depth contour which defines the landward boundary of the coastal upwelling regime. Commercial fishermen often start their initial search along the 100-m contour where experience indicates there is a high probability of finding fish. Some fishermen may also cue on surface evidence for upwelling, such as a marked change in the sea surface temperature or oceanic "colour." Smaller concentrations of fishboats were found nearshore, on Swiftsure Bank, or near islands and coastal prominences, where the interaction of tidal currents with local bottom topography can induce strong vertical mixing (Thomson and Wilson 1987; Healey et al. 1990). Although our radar-based vessel mapping program does not keep track of individual fishboats, the especially large concentration of vessels west of Tofino presumably represents day boats fishing out of this primary fishing port.

Thermal variance distributions derived from satellite thermal imagery indicate that a majority of fishboats, from the coast to water depths of 1000 m, fish in regions of low surface thermal variance. However, the exact relationship between fishboat distribution and thermal variance appears to be more complex than that proposed by Healey et al. (1990). In particular, the highest concentrations of fishboats in July 1988 were found at low to moderate variance levels of $4\text{--}6(^{\circ}\text{C})^2$ rather than at the lowest variance levels as suggested by Healey et al. (1990). The July 1988 distribution was presumably representative of the thermal variances over the middepth shelf range (61–100 m) within the transition zone formed between the northwestward flowing coastal current and southeastward flowing shelf-break current.

The one cluster of midshelf fishboats we were able to survey in detail (cluster 2, see Fig. 3) was coincident with a cyclonic eddy-like feature formed along the inshore boundary of the shelf-break current. This finding corroborates the hypothesis that clusters of fishboats are linked to locally intensified upwelling. The July 1988 results extend the link between fishboat aggregation and upwelling from the scale of the prevailing circulation (≈ 100 km) to the scale of isolated cyclonic eddies (≈ 10 km). The high number of acoustic targets in fishboat cluster 2 confirmed that it was a zone of high fish concentration.

Alongshore mesoscale variability in upwelling intensity is a common feature of coastal upwelling regimes (Brink 1983) where the separation between upwelling zones is characterized by the internal deformation radius, $r = NH/f$, where N is the Brunt-Väisälä frequency ($N^2 = -g/\rho \, dp/dz$), H is the depth scale, ρ is the water density, and f is the local Coriolis parameter. According to Ikeda et al. (1984), Thomson (1984), and Thomson and Gower (1985), eddy-like features over the outer portion of the Vancouver Island continental shelf originate with baroclinic instability in the longshore flow and, once formed, persist for periods of about 10 d to several weeks. These features clearly have a persistence sufficiently long to attract aggregations of fish and fishermen. Over the Vancouver Island shelf, $r \approx 25$ km, which is roughly the alongshore distance between the major vessel clusters observed during July 1988 (Fig. 3).

Although Swiftsure Bank is a traditional fishing ground whose sustained productivity is presumably linked to the physical environment, the connection between the circulation and the concentration of fishboats and acoustic targets over the bank (cluster 5, Fig. 3) differed from that observed near midshelf (cluster 2). It is possible that the high concentration of vessels was related to an eddy retention mechanism for fish similar to that proposed by Freeland (1988) or to the presence of weak flow convergence over the bank (M. Foreman, DFO, Institute

of Ocean Sciences, Sidney, B.C., pers. comm.) that helps concentrate plankton and their predators. The latter notion is supported by the weak geostrophic flow observed on the north side of the bank (Fig. 9B). Because the top of the bank is shallow (<50 m depth) and biological productivity is not limited by nutrients in this region (cf. Crawford and Dewey 1989), it also is possible that the salmon are feeding on large stocks of zooplankton or small fish living in weak currents within the bottom boundary layer. The last possibility is attractive in the sense that chinook salmon often represent a high percentage of the catch over Swiftsure Bank (Healey 1986) and this species is usually found near bottom over these banks.

If we are correct in assuming that the fishermen adjust to changes in salmon distribution and fish where concentrations of salmon are high, then the observed distribution of fishboats indicates a strong link between fish aggregation and circulation over the continental shelf. On a regional basis, fishboats tend to cluster within the ridge-like transition zone and within nearshore regions of locally intensified tidal mixing such as promontories and shallow banks. Alignment of the fishboats within the 90- to 110-m depth contours is seen to arise directly from the controlling effect of topography on the current and upwelling, and hence on the distribution of fish. The transition zone marks the shoreward boundary of the wind-induced upwelling domain and is a region of weak and poorly organized mean flow (Thomson et al. 1989). Because the shelf-break regime is known to contain a large zooplankton biomass (Simard and Mackas 1989), salmon may take advantage of the nearby food source while minimizing the amount of energy needed to swim against the wind-forced near-surface currents.

Fishboats (and presumably salmon) concentrate in distinct and slowly changing clusters within the transition zone. The roughly 10-km separation between major vessel clusters is consistent with the internal (baroclinic) deformation scale, NH/f , for mesoscale eddies and meanders in the region. Examination of the detailed water property structure in the vicinity of the fishboat cluster within the northern CTD grid for July 1988 revealed that the vessels were concentrated in a locally intensified upwelling center. An adjacent cluster (cluster 1) was also within an upwelling center, as revealed by the broader scale oceanographic survey (compare Fig. 3 and 5B). These results suggest that fishboats in the transition region aggregate within "hot spots" associated with cyclonic, upwelling eddies formed in response to local wind forcing or as transient responses to baroclinic instability and meandering of the mean flow. In contrast, fishboat aggregations within the nearshore domain are more closely tied to local bathymetric features or to increased vertical mixing near promontories. Oceanographic mechanisms that might account for vessel (and fish) aggregations on Swiftsure Bank are less clear but apparently differ from those influencing vessel aggregations within the midshelf transition zone.

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Responses of Immature Blackflies (Diptera: Simuliidae) to Experimental Pulses of Acidity

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An investigation to determine the responses of immature blackflies to experimental pulses of acidity simulating snowmelt episodes was conducted. Five species of mature blackfly larvae were subjected to 3- or 4-d experimental pH depressions to approximately 4.0 or 3.5 in stream channels. Differences among species in response to acidity were observed. All species except *Simulium vittatum* and possibly *S. venustum* were able to tolerate pH depressions to approximately 4.0, while only *Cnephia dacotensis* and possibly *S. decorum* were able to tolerate pH depressions to approximately 3.5. The survival of the larvae (only late instars were tested) was significantly affected by the acidity, while pupal survival generally was not. The rate of pupation, however, was decreased in the acidified channels, resulting in decreased emergence there also. More abnormally formed pupae developed in the acidified channels, suggesting that pupation following snowmelt ensures survival of these species.

On a effectué une étude visant à déterminer les réponses des mouches noires immatures à des changements bruts d'acidité simulant les périodes de fonte des neiges. Cinq espèces de larves matures de mouches noires ont été soumises à des baisses expérimentales de pH d'environ 4,0 à 3,5 dans des chenaux, pendant 3 à 4 jours. On a observé des différences de réponses d'une espèce à l'autre. Toutes les espèces sauf *Simulium vittatum* et possiblement *S. venustum* toléreraient des diminutions de pH d'environ 4,0, alors que seule *Cnephia dacotensis* et possiblement *S. decorum* pouvaient tolérer des diminutions de pH allant jusqu'à 3,5. La survie des larves (seuls les derniers stades larvaires ont été testés) était modifiée de façon significative par l'acidité, alors que celle des pupes ne l'était généralement pas. Toutefois, la vitesse de pupation était diminuée dans les chenaux acidifiés, ce qui entraînait là aussi une diminution de l'émergence. Un plus grand nombre de pupes anormalement formées était observé dans les chenaux acidifiés, ce qui suggère que la pupation après la fonte des neiges permet la survie de ces espèces.

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The release of sulphur and nitrogen oxides into the atmosphere by the burning of fossil fuels has been designated the major cause for increased hydrogen ion content of precipitation in recent years (Galloway et al. 1976; Likens et al. 1979). Furthermore, this increased acidity of atmospheric deposition, both wet and dry, has been linked to the corresponding acidification of freshwater systems in Finland (Haapla et al. 1975), Norway (Gjessing et al. 1976; Wright et al. 1976; Henriksen and Wright 1977), Sweden (Almer et al. 1974; Oden 1976; Hultberg and Wenblad 1980), the northeastern United States (Cogbill and Likens 1974; Hendry et al. 1980), and eastern Canada (Beamish 1976; Conroy et al. 1976; Jeffries et al. 1979). The most pronounced pH depressions in stream ecosystems occur as a result of hydrogen ion pulses during rain storms and especially during snowmelt. With the onset of snowmelt runoff, stream pH levels have dropped from circum-neutral to as low as 3.9 in Ontario (Harvey 1980). These reductions can occur within hours and may alter benthic communities long before chronic acidification has been detected (Servos and Mackie 1986).

The effects of lowered stream pH on most stream biota are well documented, including such groups as mayflies, stoneflies, caddisflies, and chironomids (e.g. Bell 1971; Sutcliffe and Carrick 1973; Hall et al. 1980; Raddum and Fjellheim 1984; MacKay and Kersey 1985; Hall and Ide 1987), but our knowledge of the effects of episodic pH depressions on blackflies (Diptera: Simuliidae) is decidedly scarce. Some studies have indicated that blackflies may be more tolerant of increased acidity than many of their stream cohabitants (Simpson et al. 1985; Sharpe et al. 1987; Hall et al. 1980; Bernard et al. 1990; Hopkins et al. 1989). In particular, Chmielewski (1989) reported an increase in blackfly emergence with increased acidity over a 50-yr time span. Furthermore, MacKay and Kersey (1985) reported abundant blackflies in acid streams in the Dorset, Ontario, area, and Hall et al. (1988) showed that *Prosimulium fuscum/mixtum* complex (Fries) was not affected when exposed to short-term pulses of acidity in experimental transplants near Dorset. However, only a few studies have identified blackflies to species (Stoner et al. 1984; Simpson et al. 1985; Hall et al. 1988), and none has indicated toxicity responses at the specific level. Therefore, a question of interest is how do blackfly immatures respond to short-term pulses of acidity similar to those encountered during snowmelt? Based on past surveys, the hypothesis to be tested in this research is that many

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blackfly species will be able to withstand short-term decreases in pH. Varying tolerances to low pH, explained in part by different life history strategies, are expected at the species level.

Methods

The ability of different blackfly species to survive snowmelt acid pulses was tested by subjecting larvae to simulated snowmelt pH depressions. The artificial stream channels built by Servos and Mackie (1986) and located adjacent to the outflow

of Plastic Lake, Haliburton County, Ontario (45°11' lat., 78°50' long.), were modified and used for the acidification experiments (Fig. 1).

To avoid using simuliid immatures with an acquired resistance to acidity, larvae were collected from Costello Creek, Algonquin Park, Ontario, a location that has not experienced extreme spring pH depressions (between 1984 and 1986 the maximum spring pH depression was 5.9, Hall and Ide 1987) and has water quality, including annual pH range, similar to Plastic Lake outflow (Findeis and Coleman Taylor 1988).

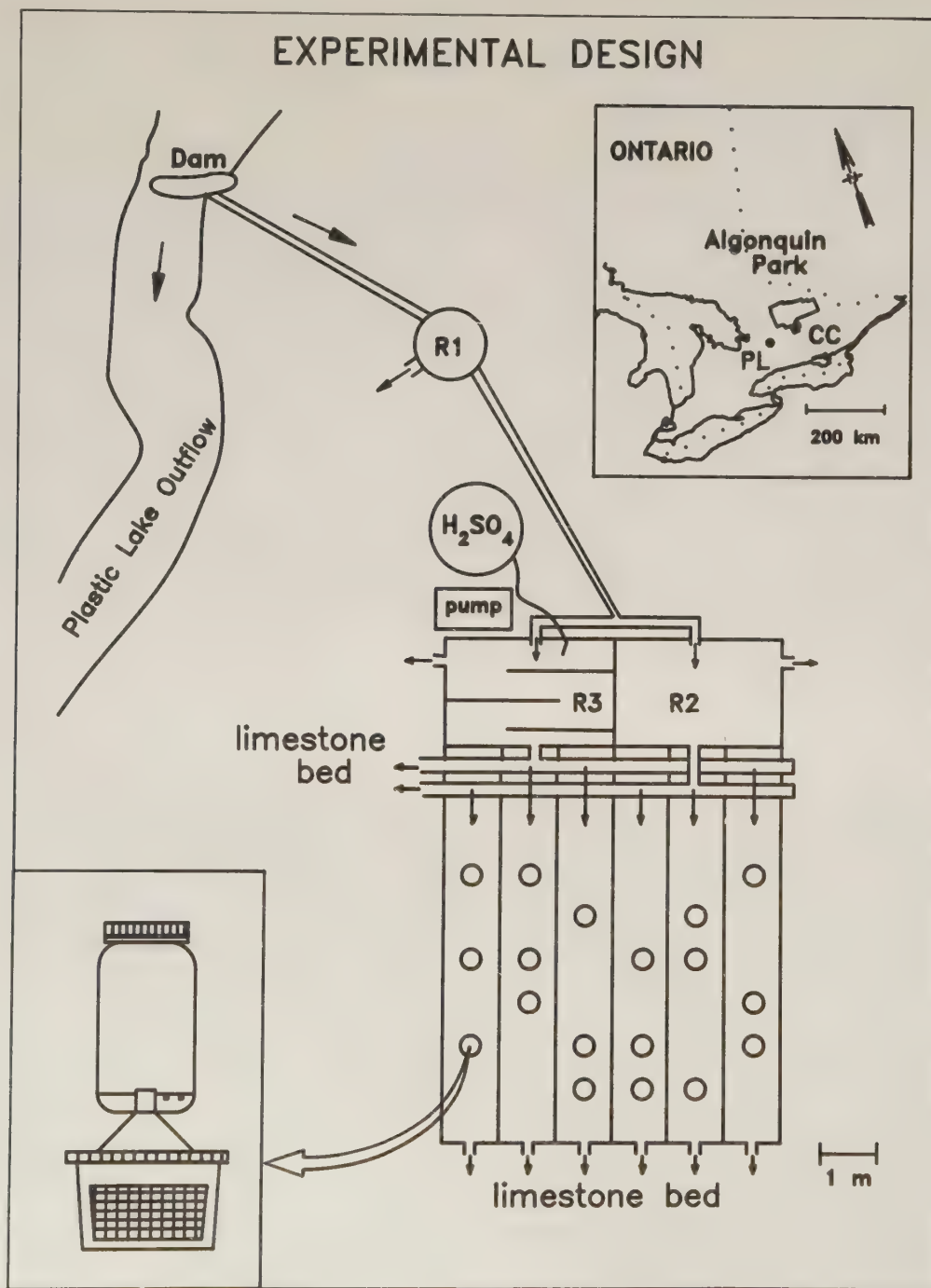


FIG. 1. Stream channels and cylindrical containers used to measure survival of larvae and pupae and emergence of adult blackflies in the experimental acidification studies. Channels were located on Plastic Lake outflow (PL) in Haliburton Co., Ontario, and blackfly larvae were collected from Costello Creek (CC), Algonquin Park, Ontario (inset). See text for details on construction and operation. Arrows indicate water flow.

TABLE 1. Experimental design. Three sets (= runs) each with two experiments were conducted. In run 2 the experiment at pH 3.5 was discarded because of technical problems. Six channels (3 acidified, 3 control) $\times$ 3 chambers/channel $\times$ 50 blackfly larvae/emergence chamber = 900 larvae total per experiment.

Run	Starting date, 1987	Duration (d)	Target pH, acidified channels	pH (SE) control channels
1	29 April	4	4.0	5.3 (0.6)
	4 May	4	3.5	5.7 (0.1)
2	19 May	3	4.0	5.8 (0.0)
	22 May	4	3.5	5.8 (0.0)
3	12 June	3	4.0	5.8 (0.0)
	15 June	3	3.5	5.9 (0.0)

A small concrete dam was constructed across Plastic Lake outflow with a 10-cm A.B.S. plastic overflow pipe leading to reservoir 1(R1, approximately 20 L) 5 m downstream and then to the wooden plasticized channel system 5 m farther downstream (Fig. 1). Here, the pipe divided, filling two larger reservoirs (R2 and R3) of approximately 700 L each. All three reservoirs had overflows. R3 was acidified by mixing 1 N sulphuric acid with stream water via a peristaltic pump. This reservoir had a mixing cup and baffles to ensure complete mixing of the acid. R2 contained a reference supply of stream water. One 7.3-cm manifold from the control and one from the acid reservoir supplied three flow-through channels (each 3.1 m $\times$ 28 cm $\times$ 45 cm). Water depth was approximately 5 cm. Mean discharges for the three experimental runs were approximately 120, 160, and 390 mL/s, respectively. Acidified and control channels were chosen randomly and were different for each experiment.

Chambers were designed to contain larvae in the stream channels while allowing water to flow unimpeded through the channels (Fig. 1). Two rectangular holes were cut from opposite sides of 500-mL cylindrical, plastic containers and replaced with Nitex netting (430- μ m mesh for the first experiment and 250- μ m for the second and third). To catch emerging adults, a 6-mm funnel was inverted and glued to a lid with a circular hole of the same size. The small end of the funnel protruded through a hole cut in the bottom of a 500-mL Nalgene bottle and was also glued. The emergence chamber was completely sealed so that emerging adults would fly through the funnel and drop into the Nalgene bottle containing 70% alcohol.

Rocks, sticks, and trailing vegetation with attached larvae were taken from all areas of Costello Creek to collect the most diverse selection of species as possible. They were then transferred to aerated coolers filled with stream water. Larvae were carefully transferred to emergence chambers (50 per chamber) with forceps suspended with a drop of water between its arms and then placed into wooden streamside channels within 3–4 h. Larvae greater than approximately 0.7 cm long were selected because smaller larvae could escape through the mesh of the chambers. Larvae were not identified at this point to minimize handling effects. Species distribution among the chambers was not known but assumed to be random.

Three runs of two experiments each were conducted (Table 1). Starting dates were chosen on or before peak emergence for the greatest number of species, based on 11 yr of emergence data from Costello Creek (Davies 1950; Hayton 1979). Runs 1, 2, and 3 were started on 29 April, 19 May, and 12 June 1987, respectively. The first run consisted of 4 d at pH 4.0 and 4 d at pH 3.5, the second consisted of 3 d at pH 4.0

and 4 d at pH 3.5, and the third consisted of 3 d at pH 4.0 and 3 d at pH 3.5.

At the beginning of each experiment, three emergence chambers were randomly placed in each of six channels (6 channels $\times$ 3 chambers/channel $\times$ 50 larvae/chamber = 900 larvae). Larvae were allowed to acclimate overnight in the channels. Dead larvae were removed the following morning and discarded. The pH was then decreased to 4.0 or 3.5 in treatment channels within 2 h and maintained at that level until the end of the experiment. The chambers were checked daily and dead blackflies were removed, counted, and preserved in 95% ethanol. At the end of each experiment the remaining emergence chambers were removed from the channels and the live and dead blackflies preserved.

Back-transplants back to Costello Creek were initiated to determine whether handling had adverse effects on blackfly survival. Six emergence chambers (50 larvae in each previously collected from Costello Creek) were returned to Costello Creek on the same day of the first collection of runs 2 (19 May) and 3 (12 June). Three plastic pails with large-mesh (approximately 1 cm) screened walls on opposite sides of the pails were placed in the stream and two emergence chambers placed in each. Both chambers were checked for live and dead larvae when the second collection per run was made (3 d later). One chamber was removed at this time and the live and dead blackflies counted. Another chamber containing blackflies that had been collected that day and returned to the creek from the laboratory was placed in each pail. Both chambers were left until the end of the run (3 d more) when larvae were removed and counted.

The temperature and pH of Costello Creek were taken at the time of each collection, and maximum and minimum stream temperatures were recorded daily. pH of one control channel and one acidified channel was monitored at least hourly during the day and bi-hourly overnight twice during run 3. Discharge of each channel was checked on a regular basis (usually once per day), except in run 2 when it was recorded only once. Discharge was measured by catching the entire flow of a channel in a graduated cylinder for 10 s. Water samples (one from each channel) were taken at the beginning and end of each run for chemical analysis of pH, alkalinity, total aluminum, conductivity, calcium, dissolved inorganic carbon, dissolved organic carbon, and sulphate. At the end of each run, water samples were taken (from one acidified channel and one control channel) for analysis of cadmium, copper, lead, and zinc. All water samples were analyzed at the Ontario Ministry of the Environment Research Centre, Dorset, Ontario, following standard procedures (LaZerte 1984; Locke and Scott 1986).

Blackflies were identified using keys of Davies et al. (1962) for the adults and Wood et al. (1963) for the larvae and pupae. No attempt was made to separate the species cytotypically. Consequently, the names used refer to morphospecies only. At time of identification, the head capsule of each larvae was measured at its widest dorsal point using an ocular micrometer (accuracy of 0.025 mm).

Due to variation in conditions among runs, each experiment was analyzed separately with qualitative comparisons made between experiments. The arsine-transformed, unpooled survival data, weighted for number of larvae of each species in an emergence chamber, were analyzed with a nested analysis of variance (ANOVA) to determine among-chamber variability in survival within each species. Since the among-chamber variation in survival was not significant ($P = 0.10$ – 0.94), the numbers of live and dead larvae of each species were pooled for all emergence chambers within an experiment. Subsequently, con-

TABLE 2. Percent survival of *Cnephia* and *Simulium* spp. in the acidification experiments. Survival was pooled over all emergence chambers in the control or acidified channels. Contingency table (2×2) chi-square probabilities are given (*significantly different survival in the control and acidified channels at $\alpha = 0.05$).

Experiment	Species	Percent survival (N)		χ^2 probability
		Control	Acidified	
Run 1, pH 4.0	<i>C. dacotensis</i>	59.8 (122)	51.1 (94)	0.1–0.5
	<i>S. venustum</i>	47.4 (215)	29.5 (261)	<0.001*
Run 1, pH 3.5	<i>C. dacotensis</i>	43.1 (130)	41.3 (80)	>0.9
	<i>S. decorum</i>	44.4 (18)	20.0 (10)	0.1–0.5
Run 2, pH 4.0	<i>S. decorum</i>	71.4 (7)	25.0 (16)	0.1–0.5
	<i>S. venustum</i>	75.6 (123)	71.8 (231)	0.5–0.9
	<i>S. verecundum</i>	92.9 (85)	89.1 (101)	0.5–0.9
Run 3, pH 4.0	<i>S. decorum</i>	42.3 (156)	42.1 (126)	>0.9
	<i>S. venustum</i>	40.6 (106)	26.3 (136)	0.025–0.05*
	<i>S. vittatum</i>	40.3 (77)	18.9 (90)	0.001–0.005*
Run 3, pH 3.5	<i>S. decorum</i>	50.8 (65)	6.0 (84)	<0.001*
	<i>S. venustum</i>	59.5 (76)	2.5 (157)	<0.001*
	<i>S. verecundum</i>	79.3 (62)	39.1 (46)	<0.001*
	<i>S. vittatum</i>	49.1 (108)	8.0 (100)	<0.001*

TABLE 3. Chemical analysis of water samples from the experimental acidification channels. Data represent arithmetic means (with standard deviations in parentheses) of samples from three acid or three control channels at the start and end of each month. Cd, Cu, Pb, and Zn were measured on water collected from one randomly chosen acidified and control channel at the end of each run. "<" indicates a value at the minimum detection level.

Run	Channel	Sample	pH	Alkalinity (mg/L)	Aluminum (μ g/L)	Calcium (mg/L)	Conductivity at 25°C (μ S/cm)	DIC (mg/L)	DOC (mg/L)	Sulphate (mg/L)	Cad- mium (μ g/L)	Cop- per (μ g/L)	Lead (μ g/L)	Zinc (μ g/L)
1	Control	Start	5.31 (0.63)	-0.34 (1.01)	27.33 (0.58)	1.84 (0.03)	21.17 (0.06)	0.53 (0.01)	1.83 (0.32)	6.00 (0.22)	0.008	<0.30	<0.04	5.26
		End	5.65 (0.08)	0.19 (0.05)	28.67 (2.31)	1.81 (0.07)	21.43 (0.15)	0.60 (0.01)	1.73 (0.06)	6.30 (0.20)				
	Acid	Start	4.94 (0.07)	-0.66 (0.15)	28.33 (1.53)	1.86 (0.01)	25.63 (0.25)	0.52 (0.01)	1.60 (0.00)	6.75 (0.00)				
		End	3.51 (0.02)	-20.00 (0.00)	39.33 (13.58)	2.22 (0.14)	131.10 (61.37)	0.57 (0.01)	1.77 (0.06)	24.13 (0.25)				
2	Control	Start	5.79 (0.02)	0.52 (0.04)	19.33 (2.31)	1.63 (0.06)	20.97 (0.25)	0.61 (0.01)	1.53 (0.06)	6.10 (0.09)	0.076	<0.30	0.21	3.47
		End	5.78 (0.01)	0.49 (0.03)	24.00 (1.73)	1.73 (0.06)	21.97 (0.29)	0.76 (0.01)	1.70 (0.00)	6.16 (0.09)				
	Acid	Start	4.01 (0.01)	-5.13 (0.12)	22.33 (1.53)	2.10 (0.46)	58.10 (0.10)	0.59 (0.01)	1.53 (0.06)	9.38 (2.80)				
		End	3.59 (0.02)	-1.60 (0.10)	27.00 (1.73)	2.60 (0.35)	135.00 (3.61)	0.73 (0.01)	1.80 (0.00)	13.88 (0.84)				
3	Control	Start	5.84 (0.02)	0.53 (0.08)	18.33 (0.58)	1.73 (0.12)	21.10 (0.10)	0.66 (0.03)	1.93 (0.06)	6.18 (0.06)	<0.010	<0.30	—	3.09
		End	5.91 (0.01)	0.78 (0.03)	18.00 (0.00)	1.80 (0.10)	21.00 (0.26)	0.63 (0.01)	2.07 (0.06)	5.85 (0.00)				
	Acid	Start	4.14 (0.01)	-4.30 (0.00)	20.33 (0.58)	2.20 (0.17)	53.47 (1.08)	0.61 (0.01)	2.07 (0.06)	10.70 (0.10)				
		End	3.55 (0.01)	-15.33 (0.58)	23.67 (0.58)	2.20 (0.26)	141.33 (0.52)	0.59 (0.02)	2.23 (0.15)	22.65 (1.58)				

tingency table analysis (2×2) was performed to determine if acidification had an effect on survival of each species. Additionally, each species was subdivided into life stages, and survival of each stage was analyzed by 2×2 contingency tables. Finally, to determine if the response to acidity was the same for all species in an experiment, repeated measures ANOVA was performed on arsine-transformed, unpooled, weighted survival data. The alpha level chosen for all statistics was 0.05.

Results

Channel Conditions

The conditions in the channels were generally amenable to blackfly habitation. Larvae back-transplanted to Costello Creek had a lower survival rate than those in control channels in two out of the three acidification experiments in the artificial channels (Chmielewski 1989). Thus, it appears the control water from Plastic Lake outflow was a favourable substitute for

Costello Creek water, but the manipulations may have been a major factor in the low survival of some of the control blackflies (Table 2).

Differences in minimum and maximum temperatures of reservoir water between each run reflect a warming trend in the stream as spring progressed (Chmielewski 1989). Water temperature in run 1, which began on 29 April shortly after snowmelt, ranged between 3 and 23°C. Water temperature during run 2, which began 19 May, ranged from 10 to 18°C. The weather was much cooler and wetter in the second half of May than in the first half. During run 3 (starting on 12 June) the weather was comparatively warmer as reflected in the higher range of reservoir temperature (11–26°C).

Discharge was well replicated among channels in experiments 2 (160 mL/s) and 3 (390 mL/s). In run 1, a large part of the variation in discharge (85%) occurred between acidified and control channels, with discharge rate of control channels (128 mL/s, SE = 1.0) being higher on average (nested ANOVA, $P = 0.001$) than acidified channels (113 mL/s,

SE = 2.0). As blackflies are known to have specific flow rate requirements (Hopkins et al. 1989), the differences in flow may be, in part, responsible for differences in survival between the control and acidified channels in run 1.

A general trend during most experiments was that aluminum, calcium, sulphate, and conductivity increased more in acidified than in control channels (Table 3). Alkalinity was depressed in acidified channels of both runs 1 and 3. Observed effects of acidification on water quality were less pronounced in run 2 because of difficulty encountered in maintaining the pH at depressed levels in the second experiment of the run. Because of these difficulties the results of that experiment were discarded. Both dissolved inorganic and organic carbon increased slightly in acidified and control channels during each run (except for dissolved inorganic carbon of run 3 which decreased in both acidified and control channels, Table 3). Of the four metals measured at the end of each run (Table 3), only zinc had consistently higher concentrations in acidified channels. Copper may have been mobilized in acidified channels, and cadmium and lead were always at or near minimum detection limits.

Development and Survival of Blackflies under Acidic Conditions

Because of the random nature of the collection and distribution of the blackflies, the identity and numbers of species tested were not known until after the experiments were concluded. The results for a particular species in an experiment were not presented if the survival of the species in the control channels was less than 40% or if there were too few individuals of that species to draw reasonable conclusions. Five species were collected in sufficient numbers for meaningful analysis. These species were *Cnephia dacotensis* (Dyar and Shannon), *Simulium decorum* Walker complex, *S. venustum* Say complex, *S. verecundum* Stone and Jamnback complex, and *S. vittatum* Zetterstedt complex (Table 2).

Presently, there are no reliable characters to separate pupal stages of *S. tuberosum* (Lundström) complex, *S. venustum*, and *S. verecundum* (Stone and Jamnback 1955; Davies et al. 1962). In addition, in this study, there were morphological intergrades between the larvae of 24 *S. venustum* and *S. verecundum*. Although the numbers of these inseparable individuals were relatively few, they were presented as a separate group for the sake of completeness. This group may include pupae of *S. tuberosum* (because small numbers of the species were present) but is likely composed mostly of *S. venustum*, as this was otherwise the most numerous of the three species.

Several patterns emerged from contingency table analysis of the acidification experiments (Table 2). The first was that survival of one species, *C. dacotensis*, was unaffected for 4 d at either pH 4.0 or 3.5 (run 1). The second pattern, exhibited by *S. verecundum*, was that pH 4.0 had no effect on survival for 3 d (run 2) and pH 3.5 caused reduced survival (run 3). A third response was significantly reduced survival at both pH levels for 3 d, as exhibited by *S. vittatum* (run 3). Finally, *S. venustum* and *S. decorum* yielded somewhat conflicting results. At pH 4.0 the survival of *S. venustum* was unaffected in one 3-d experiment (run 2) but reduced in another 3-d experiment with borderline significance (run 3) in one 4-d experiment (run 1). At pH 3.5 for 3 d, its survival was significantly reduced in the acidified channels (run 3). The survival of *S. decorum* was unaffected at pH 4.0 for 3 d (runs 2 and 3). At pH 3.5, its survival was unchanged when subjected for 4 d

TABLE 4. Percentages of larvae (L), abnormal half-pupated forms (1/2P), pupae (P), and adults (Ad) of *Cnephia* and *Simulium* spp. remaining of the total number (N) in the control (C) and acidified (A) channels at the end of the experimental acidifications. *Life stages with significantly greater survival in the control channels; **life stages with significantly greater survival in the acidified channels; TRT = treatment.

Species	TRT	Run 1, pH 4.0					Run 1, pH 3.5					Run 2, pH 4.0					Run 3, pH 4.0					Run 3, pH 3.5					
		N	L	1/2P	P	Ad	N	L	1/2P	P	Ad	N	L	1/2P	P	Ad	N	L	1/2P	P	Ad	N	L	1/2P	P	Ad	
<i>C. dacotensis</i>	C	122	96	1	2	0	130	100	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	A	94	100	0	0	0	80	100	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>S. decorum</i>	C	—	—	—	—	—	18	94	0	6	0	7	57	0	43	0	156	35*	8	57	1	65	52*	3	31	14	
	A	—	—	—	—	—	10	100	0	0	0	16	75	19	6	0	126	48	30	21	1	84	74	16	6	5	
<i>S. venustum</i>	C	215	92*	7	1	0	—	—	—	—	—	123	91	7	2	0	106	64*	11	25**	0	76	86*	13	1	0	
	A	261	91	9	0	0	—	—	—	—	—	231	91	9	0	0	136	81	15	4	0	157	75	22	3	0	
<i>S. verecundum</i>	C	—	—	—	—	—	—	—	—	—	—	—	85	97	2	1	0	—	—	—	—	—	62	6*	8	72	15
	A	—	—	—	—	—	—	—	—	—	—	101	97	3	0	0	—	—	—	—	—	46	39	15	35	11	
<i>S. vittatum</i>	C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	77	65*	9	26	0	108	49*	8	39	4	
	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	90	76	18	6	0	100	71	17	10	2	
<i>S. t/v/v^a</i>	C	35	8	46	46	0	16	0	50	50	0	77	18	9	73**	—	48	0	0	100	0	107	0	8	89*	3	
	A	24	0	58	42	0	8	0	38	62	0	46	4	35	61	—	36	0	39	61	0	35	0	49	49	3	

^aCombined group of inseparable *S. tuberosum*, *S. venustum*, and *S. verecundum*.

(run 1), but it was significantly affected during a 3-d experiment (run 3).

There were no differences observed when survival in acidic conditions was compared among species within an experiment. Only in the two experiments of run 3 were there representatives of each species in each emergence chamber to satisfy the requirements of the repeated measures ANOVA that there be no missing values (Hand and Taylor 1987). In the pH 4.0 experiments the results of repeated measures ANOVA did not agree completely with contingency table results. The latter indicated a significantly lower survival of *S. venustum* ($0.025 < P < 0.05$) and *S. vittatum* ($0.001 < P < 0.005$) in acidified as opposed to control channels whereas repeated measures ANOVA indicated that, among species, blackfly survival between acidified and control channels was not significantly different (although the significance level was borderline at $P = 0.0562$). Accordingly, since no species was significantly affected by acidity at pH 4.0, repeated measures ANOVA found no significant differences in tolerances to acidity among species ($P = 0.4147$). Alternatively, at pH 3.5 in run 3 the results of repeated measures ANOVA agreed with results of contingency tables in that among species there was a significant difference in survival between those in control and acidified channels ($P = 0.0001$). Furthermore, repeated measures ANOVA also indicated that there was no difference among species in the increase in mortality correlated with acidity ($P = 0.3532$).

During the experiments the blackfly larvae matured at various rates (Table 4). By the end of each experiment the majority of blackflies were still in the larval stage, with the size of the majority increasing with each successive experiment. ANOVA followed by Tukey's multiple range tests on the larval head capsule widths also indicated that larval size increased in successive experiments in all species except *S. venustum* ($P = 0.0009$ for *S. verecundum*; $P = 0.0001$ for all others). It exhibited a bimodal increase, possibly indicating the presence of two generations (*S. decorum* was the only species that displayed a significantly greater mean head capsule width in the acidified channels as opposed to control channels ($P = 0.0156$)). The rate of pupation and adult emergence was greatest for most species in the two experiments of run 3 in early June.

There were three important findings on stage-dependent sensitivity to acidity (Table 4). First, if the survival of a species was found to be significantly reduced under acidic treatment, the stage that was responsible for the difference was almost invariably the larval stage. In other words, once the blackflies had pupated, the acidity did not affect their survival. In fact, pupal survival was significantly greater in acidified channels at least twice (Table 4). Second, the percentage of pupation overall was decreased in the acidified channels in all species but *S. venustum* (but many of its pupae were included in the inseparable group of *S. tuberosum*, *S. venustum*, and *S. tuberosum*). Accordingly, there was also a smaller percentage of blackflies emerging from the acidified channels. Third, many individuals that had attempted pupation in the experiments were malformed. The head and appendages were disfigured to varying degrees and the cocoon was often incomplete or missing. These "half-pupated forms" occurred most often in the acidified channels. Quite unexpectedly, however, their survival was often numerically greater (although significantly greater only once) in acidified than in control channels (see Chmielewski 1989).

Discussion

The present study has extended our knowledge of the uncommon tolerance of blackflies to reduced pH but has also indicated

that there are differences in acid tolerances among species and life stages (larvae, pupae, and adult) of blackflies. The survival of several species of blackflies at a pH of approximately 4.0 in the present study is in agreement with other studies that have found blackflies to be more acid tolerant than other taxa. Surveys of acidified and nonacidified streams have revealed a distinct tolerance of blackflies to depressed pH relative to other stream invertebrates (Minshall and Minshall 1978; Sutcliffe and Carrick 1973; Stoner et al. 1984; Simpson et al. 1985; Sharpe et al. 1987). More specifically, *Simulium* spp. drift rates were not affected in a 12-h whole-stream acidification experiment from a pH of 7.0 to 5.9 whereas drift rates of other taxa increased markedly (Bernard et al. 1990). *Simulium* spp. drift rates were also found to be unaffected by 6- to 8-h depressions of pH to approximately 5.0 in naturally colonized stream channel experiments (Cooper et al. 1988; Hopkins et al. 1989). Even in a study where individuals of *P. fuscum/mixtum* complex were transplanted from streams of pH 6.2 to 4.5 for 4 d, survival was nearly complete (Hall et al. 1988). In the same study in a 10-d transplant from a stream of pH 5.6 to one of 4.5, an 80% survival rate for blackflies was obtained (Hall et al. 1988). It was not until stream pH was depressed to 4.0 for 5 mo that a definite negative response to acidity was detected in increased *Prosimulium* spp. drift (Hall et al. 1980). A similar pH depression to 4.0 in the present study also elicited a negative response in *S. vittatum* after 3 d, but at least three other species, *C. dacotensis*, *S. decorum*, *S. verecundum*, and possibly *S. venustum*, were unaffected at this pH level. Further testing of the blackflies revealed even more specific differences, as *C. dacotensis* and possibly *S. decorum* were the only species able to tolerate a pH reduced to approximately 3.5. Because of the differences found among species in their response to acidity, the importance of species level identification in invertebrate toxicity studies cannot be stressed enough.

Smaller size classes of other invertebrates are often the most sensitive to experimentally lowered pH levels (Allan and Burton 1986; Allard and Moreau 1987). Depressed emergence of stream invertebrates exposed to decreased pH has also been observed (Bell 1971; Hall et al. 1980; Zischke et al. 1983). Zischke et al. (1983) and Bell (1971) concluded that the emerging insect is the most sensitive stage, but their conclusions may have been premature. In stream channel acidification studies where organisms were allowed to colonize channels naturally (Zischke et al. 1983), it was impossible to monitor survival of early stages. Bell (1971) used only mature larvae of stream insects in his laboratory bioassays and therefore should not have concluded that the emerging insect was the most sensitive stage without having tested early stages. Unfortunately, smaller blackfly instars could not be included in this study either because smaller mesh sizes could not be used due to problems with rapid siltation. Thus, no definitive conclusions can be made as to which life stage is the most sensitive. However, some insights into stage-dependent sensitivity of blackflies are offered. Although there were little emergence data in these studies, there was a slightly decreased emergence observed in some species in acidified channels, results which support those of Bell (1971), Hall et al. (1980), and Zischke et al. (1983). However, observation of decreased pupation of some species in acidified channels (*C. dacotensis*, *S. decorum*, *S. venustum/verecundum*, *S. vittatum*, Table 4) leads to the more precise conclusion that, because fewer blackflies pupate in acidified conditions, emergence may be decreased.

To date, no one has attempted to rear blackflies in outdoor stream channels. Difficulty in raising blackflies has been attrib-

uted to susceptibility of stream insects to accumulated ammonium wastes, and narrow hydrochemical temperature, current, and food tolerances (Edman and Simmons 1985). However, it is unclear why 3- or 4-d survival of control blackflies in this study was sometimes low, compared with laboratory studies lasting up to several months (Edman and Simmons 1985), although it is possible that the blackfly mortality observed in control channels and back-transplants may be more similar to their natural mortality during development and emergence. There are several factors that may have influenced survival. Water temperature has been shown to be a critical factor affecting larval blackfly distribution (Ross and Merritt 1978) and may not have been optimal during rearing, especially for some of the early spring species that prefer lower temperatures. Their food requirements appeared to be satisfied, as their guts were always full. Although there are no indications of such, perhaps there was a critical difference that was not monitored between water from Plastic Lake outflow and Costello Creek. Lowered rates of discharge have also been found to be an additional stress confounding interpretation of results from acidification testing of blackflies (Hopkins et al. 1989). It may not have been optimal for all species at all times but, more importantly, the variation in discharge between acidified and control chambers in run 1 may have been, in part, responsible for the significantly lower survival of *S. venustum* in the acidified channels. *Cnephia dacotensis* did not seem to be affected in the same experiment. Perhaps the most important point to consider is that since there were three to five species being raised simultaneously, it was impossible to meet all of their specific requirements. However, any advantages one species could have had over another were taken into account by repeated measures ANOVA because it was difference in survival between control and treated of each species that was compared, not just survival of the treated.

The increased number of malformed pupae in acidified channels clearly indicates presence of a severe physiological stress on blackflies when subjected to depressed pH levels. No studies have assessed direct toxicity of excess hydrogen ions on immature blackflies; thus, it is difficult to point out the exact physiological causes of malformations or of mortality. However, Hall et al. (1988) have monitored cation concentrations of blackflies cross-transplanted from a site at pH 6.5 to one of pH 4.2. They reported a significant decrease in whole-body calcium and sodium concentrations, which may indicate a change in ionoregulation with decreased pH (Hall et al. 1988). The occurrence of abnormal, half-pupated forms is thought to be the result of less than optimal rearing conditions.

The stage at which a species is present when stream pH depressions occur is an important aspect of life history strategies (see Ross and Merritt 1987). Most of the blackflies tested from Costello Creek overwinter in the egg stage (Davies et al. 1962), and hatching does not occur until mid-April or later in most species (Davies et al. 1962), generally after snowmelt. Only a few early spring species may overwinter as larvae, including *C. dacotensis* and *S. vittatum*, although there are conflicting reports for both (see Hayton 1979). These are the only species of blackfly included in this study whose larvae may have experienced spring snowmelt. More importantly, those tested were survivors of the real snowmelt, and *C. dacotensis* is deemed the species (of those tested) most tolerant to pH depressions. Perhaps because the majority of blackflies in temperate zones do overwinter as eggs and miss snowmelt as larvae, they are more tolerant than other taxa that may overwinter as larvae. The generally high larval survival in acid-

ified channels in this study suggests that blackfly larvae may be able to survive pH depressions following rain events subsequent to snowmelt. Since the pupation process itself appeared to be negatively affected by low pH, insects surviving in areas of high acidic deposition need to delay pupation until after snowmelt. In contrast, those species that are more tolerant to pH depressions can occupy early-season habitats that are not amenable to more pH-sensitive species. Our hypothesis, then, that many blackfly species will be able to withstand short-term decreases in pH was supported by data for some species and refuted based on others. Life history strategies clearly play a dominant role.

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